



NOAA Office of Ocean Exploration Quick Look Report

Expedition Title: "Discovery of New Resources with Pharmaceutical Potential in the Gulf of Mexico, Straits of Florida, and South Atlantic Bight"

Results (please check all disciplines in which this cruise collected data)	Details (please describe any novel discoveries in the discipline, answers such as "possible, awaiting data analysis" and "no apparent discoveries" are acceptable)
Bathymetric Mapping X Yes <input type="checkbox"/> No	(please note total area mapped and technology employed, e.g. multibeam, side scan, etc.) SEABEAM Maps- 1) 9/11/03, SW Florida Shelf, 1988 Trawl Site, 250'; 2) 9/11/03, SW Florida Shelf, Lithoherm Site, 1827'; 3) 9/13/03, NW Florida Shelf, Twin Ridges, 243'; 4) 9/14/03, NW Florida Shelf, Madison & Swanson MPA, 270'; 5) 9/17/03, S. Alabama, Mississippi Canyon Block 118, 3000'
New Species Discovered X Yes <input type="checkbox"/> No	(please note number, type, and significance, i.e. radically new vs. slight adaptation of known species) Taxonomy in progress
Bio-prospecting X Yes <input type="checkbox"/> No	(please note number, type, and potential use of new compounds discovered) Biochemical work in progress
Habitat Range Extended <input type="checkbox"/> Yes <input type="checkbox"/> No	(please note species discovered in new habitats and how far from previous range were they found) Taxonomy in progress
Chemical Processes X Yes <input type="checkbox"/> No	(please note new or unusual chemical properties such as methane seeps, hypersaline pools, vents, etc. observed) Gas seep observed at Lithoherm site; Dead and live bivalves and Vestimentiferan worms present at deep water lithoherm sites indicating potential chemosynthetic communities.
Geologic Processes X Yes <input type="checkbox"/> No	(please note new or unusual geologic processes that may impact scientific understanding of the region) First video and ROV dive on SW Florida Shelf Lophelia lithoherm. Adds new data to 1987 publication by Newton et al. which described the site from seismic profiles and dredge samples only. Considerable dead thickets of Lophelia.
Physical Processes X Yes <input type="checkbox"/> No	(please note new or unusual oceanographic processes that may impact scientific understanding of the region) First ROV dive on deep water Lithoherm site off SW Florida shelf, providing new data on physical bottom conditions.
Sub/ROV/AUV Dives X Yes <input type="checkbox"/> No	(please note name, type, and cumulative hours of bottom time for each platform / if available please provide average working time per dive for each platform / please note if new depth records were set) SONSUB ROV- 22 dives, 87.72 hours of dive time; 1 scuba dive site (5 hours dive time)
New Technology X Yes <input type="checkbox"/> No	(please note any new tools developed for or during this cruise, also identify first use of an existing technology in a new application) SONSUB ROV Tools developed and fabricated for NOAA OE expeditions: 1) suction device, 2) clam shell grab, 3) large basket, 4) collection buckets for TMS
Maritime Cultural Heritage X Yes <input type="checkbox"/> No	(please note discoveries impacting knowledge of the past, i.e. number and type of shipwrecks) Apparent 19 th century anchor videotaped at Pinnacles Site, 322', but could not find after 2 hour search due to poor tracking/ navigation.
Outreach X Yes <input type="checkbox"/> No	(please describe outreach channels, e.g. web, port call, etc., used in this project) 1) NOAA OE Web site- Provided 7 biosketches, factoids, 10 essays, 12 daily logs; 2) HBOI web site connection to NOAA site; 3) Port call, St. Petersburg, 9/8/03- dockside media event, live tv and interviews; 4) Naples News- article, 9/28/03; 5) National Public Radio- 'The Florida Environment', Interview, J. Reed, 9/5/03; 6) National Public Radio- Jill Roberts, Interview, J. Reed, 9/5/03; 6) NASA Oceanography Outreach and OceanAge Project- Andrea McCurdy film interview with J. Reed and S. Pomponi for NOAAOE web site, 9/3/03; 7) Washington Letter of Oceanography, Vol. 37 (19)- article, 9/15/03; 8) Environmental News Network (ENN)- article, 9/5/03; 9) St. Petersburg Times- article, 9/5/03; 10) Ascribe, The Public Interest Newsletter- article, 9/5/03.

Students Involved X Yes <input type="checkbox"/> No	(please note the number and level of students on the expedition) Teacher at Sea from Eau Gallie High School- daily activities for students and provided numerous web logs.
Multidisciplinary X Yes <input type="checkbox"/> No	(please identify the formal disciplines represented in the science party) Taxonomy, chemistry, biology, biochemistry, molecular biology, aquaculture, cell culture, microbiology
Exploration of New Regions X Yes <input type="checkbox"/> No	(please note if the area of operations had been previously studied, if so please check no and approximate as slight, moderate or significant, the level of knowledge before the cruise) 1) First ROV dives and videotapes of deep water Lophelia coral lithoherm off SW Florida shelf. This site was first descibed in 1987 by Newton et al., based on seismic profiles and dredge samples. 2) First seabeam map of Mississippi Canyon site- a site selected as potential hard bottom/ coral site. ROV dive proved to be mud bottom with limited hard bottom and no hard corals.

**2003 NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
OFFICE OF OCEAN EXPLORATION MISSION**

MISSION SUMMARY- QUICK LOOK REPORT

LEG 1:

**Discovery of New Resources with Pharmaceutical Potential in the Gulf of Mexico, Straits of
Florida, and South Atlantic Bight
[Medicines from the Deep Sea: Exploration of the Gulf of Mexico]**

September 8-19, 2003

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I. ABSTRACT

This project seeks to explore currently untapped sources of new drugs that may be applied in the long term to the development of compounds used to study, diagnose or treat human diseases, including cancer, infectious diseases, diseases of the immune system, cardiovascular disease, and central nervous system disorders. Related to this primary objective is the development of alternative methods to harvesting from nature for production of bioactive compounds and the documentation of relatively unknown deep-water coral and live-bottom habitats in the Gulf of Mexico. This successful mission resulted in: i) collecting benthic invertebrates for biomedical research, ii) documenting the biodiversity of benthic communities, iii) isolating and culturing microorganisms, iv) preparing extracts of macroorganisms for bioactivity screens, v) preserving sub-samples for a molecular genetics, cell culture, microbiology, chemistry, and taxonomy programs, and vi) analyzing extracts for chemical activity. Virtually no drug discovery research has targeted the deep-water, live-bottom communities in the Gulf of Mexico, and in particular the microbial associates living in these communities. Some of the sites visited have never been dived previously and this is the first documentation of these habitats and biodiversity.

This successful leg of the NOAA Ocean Exploration mission completed a total of 22 Remotely Operated Vehicle (ROV) dives and one scuba dive by the Division of Biomedical Marine Research (DBMR) of Harbor Branch Oceanographic Institution (HBOI). A total of 12 sites were documented with 87.7 hours of ROV dive time and 5 hours of scuba dives. Dive sites included 1) shelf-edge paleoshorelines along the SW Florida shelf, NW Florida shelf ('Madison and Swanson Marine Protected Area', 'Twin Ridges'), and Alabama shelf ('The Pinnacles'); 2) deep-water *Lophelia* coral lithohierms off SW Florida slope, and Alabama shelf slope; and 3) three oil rig platforms off the Mississippi Delta. A total of 155 samples of benthic macrofauna and environmental samples were collected, cataloged and will be used for biomedical research and taxonomic studies of the biodiversity of these sites. A total of 69 sixty-minute mini-DV digital videotapes documented benthic habitats and species. Each videotape was documented with real-time annotations in an Excel spreadsheet which detailed habitat descriptions, fish and invertebrate identifications, sample collections, time, depth, latitude, longitude, digital still photo number, and notation of "best" video and photographs. A total of 584 digital still images were taken with the ROV (400 from video grabs, 184 from high resolution digital camera) and 255 high resolution digital still images were taken of the specimens in the laboratory. At five sites a SEABEAM map was made prior to the dives: 1) 9/11/03, SW Florida Shelf, 1988 Trawl Site, 250'; 2) 9/11/03, SW Florida Shelf, Lithoherm Site, 1827'; 3) 9/13/03, NW Florida Shelf, Twin Ridges, 243'; 4) 9/14/03, NW Florida Shelf, Madison & Swanson MPA, 270'; 5) 9/17/03, S. Alabama, Mississippi Canyon Block 118, 3000'.

A strong component of the expedition was in education and outreach through daily coverage on web sites (NOAA's site-

<http://oceanexplorer.noaa.gov/explorations/03bio/welcome.html>

and HBOI's site-www.hboi.edu), dock-side tv and newspaper interviews, numerous newspaper articles and radio interviews, and class development for educators.

II. KEY FINDINGS AND OUTCOMES

Over 87 hours of dive time using the SONSUB *Innovator* ROV from the NOAA Research Vessel *Ronald Brown* were conducted at 12 sites, including deep-water coral reefs, lithohierms, hard-bottom habitats, and oil rig platforms within the eastern Gulf of Mexico from the southwest Florida shelf and slope to the Mississippi Delta region. Benthic macrofauna and habitats are documented with approximately 69 hours of videotapes, 585 underwater and 255 shipboard digital still images, and 155 specimens of sponges, octocorals, scleractinian corals, bryozoans, ascidians, mollusks, vestimetiferan worms, echinoderms, and environmental (water, sediment, rock) samples. Taxonomic museum specimens, photographs, and videotapes are archived in HBOI's DBMR Museum. A copy of all videotapes, insitu and laboratory specimen photographs, and Excel spreadsheet of the video annotation log was submitted to NOAA OE on September 19, 2003. Subsamples of each specimen are frozen in DBMR's Molecular Genome Archive (preserved for both DNA and RNA extraction). Cryopreserved specimens are frozen for follow-up work on microbial isolations and invertebrate cell culture. The remainder of each specimen is frozen and is currently stored in DBMR's frozen sample repository to allow for chemical work up. Once the microbial isolation work is completed (2-6 months after the expedition), axenic cultures of the microbial isolates derived from the specimens will be archived in DBMR's Marine Microbial Collection.

KEY FINDINGS

- A deep-water *Lophelia* coral lithoherm off the SW Florida slope was documented for the first time with a high-definition topographic SEABEAM map, ROV dives, and videotapes. This site was first documented from seismic profiles and bottom dredge samples (Newton et al., 1987). Thickets of live and dead *Lophelia* coral were discovered on top of the pinnacles with the ROV.
- At five sites, a high-definition topographic SEABEAM map was made prior to the dives: 1) 9/11/03, SW Florida Shelf, 1988 Trawl Site, 250'; 2) 9/11/03, SW Florida Shelf, Lithoherm Site, 1827'; 3) 9/13/03, NW Florida Shelf, Twin Ridges, 243'; 4) 9/14/03, NW Florida Shelf, Madison & Swanson MPA, 270'; 5) 9/17/03, S. Alabama, Mississippi Canyon Block 118, 3000'.
- A total of 155 samples were collected including: 68 cnidaria, 60 porifera, 5 mollusca, 4 bryozoa, 3 annelida, 2 ascidiacea, 1 sipunculida, 1 sipunculida, and 7 environmental samples (water, rock, sediment).
- A total of 69 sixty-minute mini-DV digital videotapes documented benthic habitats and species. Each videotape was documented with real-time annotations in an Excel spreadsheet which detailed habitat descriptions, fish and invertebrate identifications, sample collections, time, depth, latitude, longitude, digital still photo number, and notation of "best" video and photographs.
- Taxonomy of invertebrates is ongoing and new species or new records of occurrence will be documented.
- Chemical profiles of extracts made from specimens of sponges and octocorals indicate that novel compounds are present in many of the samples. Biochemical testing of samples are ongoing.
- Several species exhibited antimicrobial activity, as determined by the shipboard disk diffusion assays. Microbial research is ongoing.
- Websites- NOAA's Ocean Explorer website documented real-time postings of essays, interviews, photographs, and video, from mission scientists, crew and educators, describing daily dive activities and discoveries. HBOI personnel and 'At-Sea Educator' provided the NOAA OE Web site with 10 essays, 12 daily logs, 7 biosketches, and 12 factoids.
- Education Benefits- NOAA's National Education Coordinator, HBOI's media lab videographer, and a high school teacher-at-sea provided a strong educational component to the mission, including lesson plan development for grades 6-12, daily dispatches and interviews, and post cruise presentations.
- Media Coverage- Pre- and post-cruise media events hosted regional TV and newspaper releases. Digital video highlights tape may be distributed to national markets. Outreach activities in addition to web and education included various media events and publications including: 1) Port call, St. Petersburg, 9/8/03- dockside media event, live tv and interviews with PIs Amy Wright and Shirley Pomponi; 2) *Naples News*- article, 9/28/03; 3) National Public Radio- 'The Florida Environment', Interview, J. Reed, 9/5/03; 4) National Public Radio- Jill Roberts, Interview, J. Reed, 9/5/03; 5) NASA Oceanography Outreach and OceanAge Project- Andrea McCurdy film interview with J. Reed and S. Pomponi for NOAA OE web site, 9/3/03; 6) *Washington Letter of Oceanography*, Vol. 37 (19)- article, 9/15/03; 7) Environmental News Network (ENN)- article, 9/5/03; 8) *St. Petersburg Times*- article, 9/5/03; 9) *Ascribe*, The Public Interest Newsletter- article, 9/5/03.

III. MISSION RATIONALE AND OBJECTIVES

The proposed research project specifically addresses the following NOAA OE Objectives:

- ◆ **“Marine life inventories: invertebrate, macro-organism, and micro-organisms”:** The proposed research will document the habitats and biodiversity of several unique deep-water habitats with videotapes, photographs, taxonomic museum specimens and preserved samples for a “Genomic Ark”. Many of the proposed sites have never been explored before by submersible, such as the deep-water reefs and sinkholes on the Florida shelf. Preliminary dives with the JSL submersible by our division in 1999 indicate that these are unique with high biodiversity. The seep, methane gas hydrate, and deep water reef communities in the Gulf of Mexico have yet to be tapped for their potential sources of new pharmaceutical products. We propose to collect samples of sufficient size to conduct biological and chemical assays for bioactivity and compound purification. HBOI is actively pursuing alternative methods to harvesting from nature for production of bioactive compounds through chemical synthesis, microbial fermentation, marine invertebrate cell culture, molecular biology, and aquaculture; thus, our focus is on sustainable use of marine resources.
- ◆ **“Locating and mapping deep-water corals”:** On the 2002 SAB mission, we discovered several exciting deep-water coral reefs in the South Atlantic Bight. We will focus on discovery of new deep-water coral reefs in the GOM.
- ◆ **“Discovery of new ocean resources” and “Prospecting for biological and mineral resources”:** We propose to seek out, recover, isolate, and culture novel organisms from unique, extreme environments; and we propose to identify unique bioactive compounds with commercial potential associated with such marine organisms. Virtually no drug discovery research has targeted these deep vent, seep, methane gas hydrate, and deep water reef communities, and in particular the microbial associates within such communities. Prior observation by our group as well as extensive review of published reports strongly support the premise that these habitats will yield exceptional new discoveries.
- ◆ **“Provide education and outreach activities”:** During the 2002 Islands in the Stream Mission, DBMR scientists provided material for curriculum development, participated in the Shipboard Open House for students, wrote essays for the web page and daily at-sea dispatches of the mission and answered questions for students about the mission. We are currently implementing a Professional Development Workshop for teachers in South and Central Florida to familiarize them with the OE program and curriculum. We envision a similar commitment to education and outreach during the 2003 mission.
- ◆ **“Provide user-friendly databases for data dissemination”:** We will provide the OE office with research data via links with an internet-based database that will be developed in collaboration with the OE Program. This database will be user-friendly and provide immediate data dissemination regarding station and habitat descriptions, species documentation, and in-situ photographs documenting species and deep water habitats.

OBJECTIVES

Our primary objective is to explore for new marine resources by discovering novel bioactive compounds from marine organisms that have potential as pharmaceutical products or biomedical research tools. The results of studies on the bioactivity and chemistry of novel compounds

isolated from these samples may be applied in the long term to the development of compounds used to study, diagnose or treat human diseases, including cancer, infectious diseases, diseases of the immune system, cardiovascular disease, and central nervous system disorders. Related to this primary objective is the development of alternative methods to harvesting from nature for production of bioactive compounds. We are actively engaged in research on chemical synthesis, microbial fermentation, marine invertebrate cell culture, combinatorial and molecular biology, and aquaculture of bioactive marine organisms.

Our second objective, which contributes to the first, is to document the biodiversity of the deep-water habitats (emphasizing sponges, cnidarians, mollusks, annelids, echinoderms, ascidians, and microorganisms) through in-situ video and photographs, and museum specimens.

Our third objective is to facilitate educational and outreach programs using at-sea web programs, interactions with teachers/ educators, media, and data dissemination via the internet.

SPECIFIC GOALS

- 1) Collect targeted benthic invertebrates for biomedical research, using the SONSUB ROV. Document the biodiversity of the deep-water, live-bottom benthic communities with videotapes, photographs, and taxonomic museum specimens.
- 2) Isolate and ferment microorganisms that live in association with deep-water marine invertebrates.
- 3) Prepare extracts of macroorganisms and microorganisms and screen them through a panel of assays to detect compounds which may have utility in the treatment of cancer, infectious diseases, cardiovascular disease, central nervous system disorders, and inflammation. The primary focus will be on the discovery of antitumor agents.
- 4) Conduct bioassay-guided fractionation followed by structure elucidation of the active compounds.
- 5) Further evaluate the pharmacological properties of the active compounds.
- 6) Develop methods for sustainable use of biomedically important marine resources [aquaculture, cell culture, microbial fermentation, combinatorial biology].
- 7) Facilitate education and outreach activities in conjunction with NOAA's OE Program through NOAA's Ocean Explorer website (www.oceanexplorer.noaa.gov), media participation, and a teacher/education component.
- 8) Provide research data via links with an internet-based database that will be developed in collaboration with NOAA's OE Program. This database will provide immediate data dissemination regarding station and habitat descriptions, species documentation, and in-situ photographs documenting species and deep water habitats.

IV. MISSION OPERATIONS AND ITINERARY

- | | |
|---------|---|
| Sept. 7 | 1000 am- HBOI personnel drive from Fort Pierce, Florida; 1400- arrive at ship, St. Petersburg commercial docks, unload and set up lab. SONSUB is apparently delayed approximately 24 hours due to tropical storm. |
| Sept. 8 | 0900- 1100- media event at dockside with tv and newspaper interviews with principal investigators Amy Wright and Shirley Pomponi. SONSUB and C&C |

- Technology continue mobbing equipment and cables; decision to depart at midnight. Transit to first dive site approximately 120 miles sw of Tampa Bay.
- Sept. 9 SW Florida Shelf Site- On site at 1030. SONSUB not ready to dive. Launch ROV at 1400. Test dive. Initial problems include- 1) 3 chip color video camera not operational, 2) high definition digital still camera not operational, 3) navigation and tracking of sub not operational. Complete one science ROV dive. Conduct SEABEAM map at night.
- Sept. 10 SW Florida Shelf Site- 1 ROV dive.
- Sept. 11 SW Florida Shelf Site- 2 ROV dives.
- Sept. 12 SW Florida Shelf, *Lophelia* Coral Lithoherm Site- 3 ROV dives; transit to next region; conduct SEABEAM map.
- Sept. 13 NW Florida Shelf, Twin Ridges Site- 3 ROV dives; transit to Madison Swanson Site; conduct SEABEAM map.
- Sept. 14 NW Florida Shelf, Madison & Swanson Marine Protected Area Site- Meet charter boat to pick up replacement cameras and additional ROV equipment; 2 ROV dives; transit to next region.
- Sept. 15 Viosca Knoll, *Lophelia* Coral Lithoherm Site- 2 ROV dives; transit to next region.
- Sept. 16 Mississippi Delta Region, Oil Rig Platform Sites- 2 ROV dives
- Sept. 17 Mississippi Delta Region, Oil Rig Platform Sites- 1 ROV dive, 1 scuba dive; transit to Mississippi Canyon Site; conduct SEABEAM map of canyon; 1 ROV dive; transit to Pinnacles Site.
- Sept. 18 Viosca Knoll, The Pinnacles Site- 4 ROV dives; transit to Panama City.
- Sept. 19 Arrive Panama City port, offload leg 1 personnel and gear. Drive to Fort Pierce, Florida.

V. MISSION PLANNING- SITE AND TARGET SELECTION

Discovery of novel chemical compounds from deep-sea marine organisms often takes us to new and exciting locations. One of the first steps in planning such an expedition is to thoroughly review the scientific literature about a new target site. This can include reviewing surveys by environmental consulting firms; government agencies such as Minerals Management Service, National Marine Fisheries, U.S. Geological Survey; and various research institutions for literature on the biology and geology of a region. This will help us determine whether an area is worth spending precious time and funds to dive. Our research expeditions have taken us throughout the Caribbean; the eastern Atlantic to the Azores, Canary Islands, Cape Verde and western Africa; the Pacific Ocean including Galapagos Islands, Samoa, Papua New Guinea, and Australia; and the Indian Ocean to the Seychelles and Thailand. Our target organisms are often associated with hard bottom habitat and include sponges, octocorals, bryozoans, tunicates, and algae. Detailed bathymetric charts are also useful in determining whether the 'right' bottom type is present that may provide essential habitat for such benthic organisms. However, most areas that we have gone to have very little known about their deep sea environments and we have to start with our best guess.

2002 Deep-Sea Discoveries

One example of discovery and exploration of the unknown deep sea occurred last year when we participated in NOAA's 'Islands in the Stream 2002 Expedition' to the South Atlantic Bight,

documenting the biodiversity of deep-water reefs on the Blake Plateau. For most of the sites that we visited with HBOI's *Johnson-Sea-Link* submersible, we were the first group to ever dive on these sites. Virtually nothing was known about the habitats and we had no idea what to expect. It was exciting to discover a variety of rich habitats with exceptional biodiversity. . On one dive we discovered a 500 ft tall pinnacle on the eastern edge of the Blake Plateau which consisted of live bushes of *Lophelia* coral, sponges, gorgonians, and black coral bushes. Several new species or new records of occurrence of sponges and octocorals were discovered. During this mission we took nearly 30 hours of video footage, 160 underwater and 508 shipboard digital still images of deep-sea marine organisms (some new to science), sampling 23 deep-water sites and collecting over 189 macroorganism samples for the drug discovery program. Subsamples of each specimen were stored in DBMR's frozen sample repository to allow for chemical analyses and studies on bioactivity. Subsamples were also frozen in DBMR's Molecular Genome Archive (preserved for both DNA and RNA extraction) and specimens were cryopreserved for follow-up work on microbial isolations and invertebrate cell culture. Cultures of ~300 microbial isolates derived from the specimens are archived in DBMR's Marine Microbial Collection. Logs of our expedition along with background information on our program and the mission summary report can be found on the NOAA OE web sites:

<http://oceanexplorer.noaa.gov/explorations/02sab/welcome.html>

http://oceanexplorer.noaa.gov/explorations/02sab/logs/summary/Leg3_summary.pdf.

Marine Targets for Biomedical Research

Over the past quarter century, more than 10,000 compounds have been reported from marine-derived organisms. These compounds encompass a wide variety of chemical structures including acetogenins, polyketides, terpenes, alkaloids, peptides and many compounds of mixed biosynthesis. A number of excellent books and reviews document the diversity of both structures and bioactivities which have been observed for marine-derived compounds (Faulkner 1984-2002, 1993; Attaway 1993; Carte 1996; Jensen 1996; Fenical 1997). At least twelve marine-derived compounds are currently under clinical investigation for their use as anticancer agents. These include: ecteinascidin from the tunicate *Ecteinascidia turbinata* which is in Phase III trials; aplidine from the ascidian *Aplidium albicans* which is in Phase II trials; dolastatin from the sea hare *Dolabella auratium* which is in Phase I clinical trials; and bryostatin from the bryozoan *Bugula neritina* which is in Phase I/II clinical trials.

Harbor Branch Oceanographic Institution (HBOI) has had an on-going drug discovery research program since 1984. Organisms have been collected throughout the world with a focus on deep water (>150 m) tropical Caribbean and Atlantic species. Research on these organisms has led to the publication of over 100 structures with over 92 patents issued. One of the more notable compounds discovered at HBOI is discodermolide a potent antitumor agent from a deep-sea sponge. The compound has been licensed to Novartis and is in Phase I clinical trials for the treatment of cancer. Other compounds of interest are the ecteinascidins, potent antitumor agents which are currently in Phase III clinical trials for the treatment of cancer. A third series of compounds are the topsentins, potent anti-inflammatory agents discovered by HBOI scientists in collaboration with Professor Bob Jacobs of UC Santa Barbara. These compounds were first isolated from the sponge *Spongosorites ruetzleri*. These compounds are under development for use additives in anti-inflammatory skin creams. A fourth series of compounds under investigation for the treatment of cancer are the lasonolides. These compounds come from the

sponge *Forcepia* which most commonly occurs in the deep-sea habitats in the Gulf of Mexico. Research to provide a large-scale supply for clinical use suggests that aquaculture and/or molecular methods may be useful in production. A multi-step synthesis has also recently been accomplished.

Gulf of Mexico Targets

Deep-water marine habitats constitute a relatively untapped resource for natural products drug discovery. Virtually no drug discovery research has targeted the deep vent, seep, methane gas hydrate, and deep-water reef communities which are accessible in this region. For all of the sites we propose to visit, our program to investigate microbial diversity associated with deep-sea invertebrates will complement our macroorganism-based drug discovery research, and provide additional opportunities for the discovery of novel compounds. Prior observations by our group as well as extensive review of published reports strongly support the premise that these habitats will yield exceptional new discoveries.

Due to available ship time, only limited work has been conducted on the biomedical potential of organisms in the Gulf of Mexico (GOM). In 1988, HBOI scientists sorted through the by-catch brought up by a commercial shrimp trawler and collected 553 samples from hard bottom habitats in the southern GOM off the southwestern Florida shelf. Surprisingly, in an NIH-funded program to discover antifungal agents, over 10% of the organisms tested from this habitat showed significant bioactivity. The active principles from these organisms have been identified and are currently under investigation. This first expedition to the GOM also resulted in the collection of a specimen of the sponge *Forcepia*, the source organism of the lasonolides (Horton, 1994). The discovery of this organism in the GOM prompted a return to the area with the *Johnson-Sea-Link* (JSL) submersible in 1999 to re-collect the sponge. Exceptional diversity of marine invertebrate species was observed and approximately ten days were spent exploring this area of live bottom. Additional productive areas were identified during this expedition but remain to be thoroughly explored. During this same expedition, two deep-water sinkholes were explored on the Pourtales Terrace and one in the SE GOM. Each was observed to have unique and diverse invertebrate populations. A specimen of the sponge *Leiodermatium* sp. collected at the GOM sinkhole site has exceptional activity in an assay which detects compounds which block the cell cycle at the G2/M interface and the active compounds are under investigation. These observations strongly suggest that exploration of additional sinkholes will provide new species for investigation.

2003 Target Sites

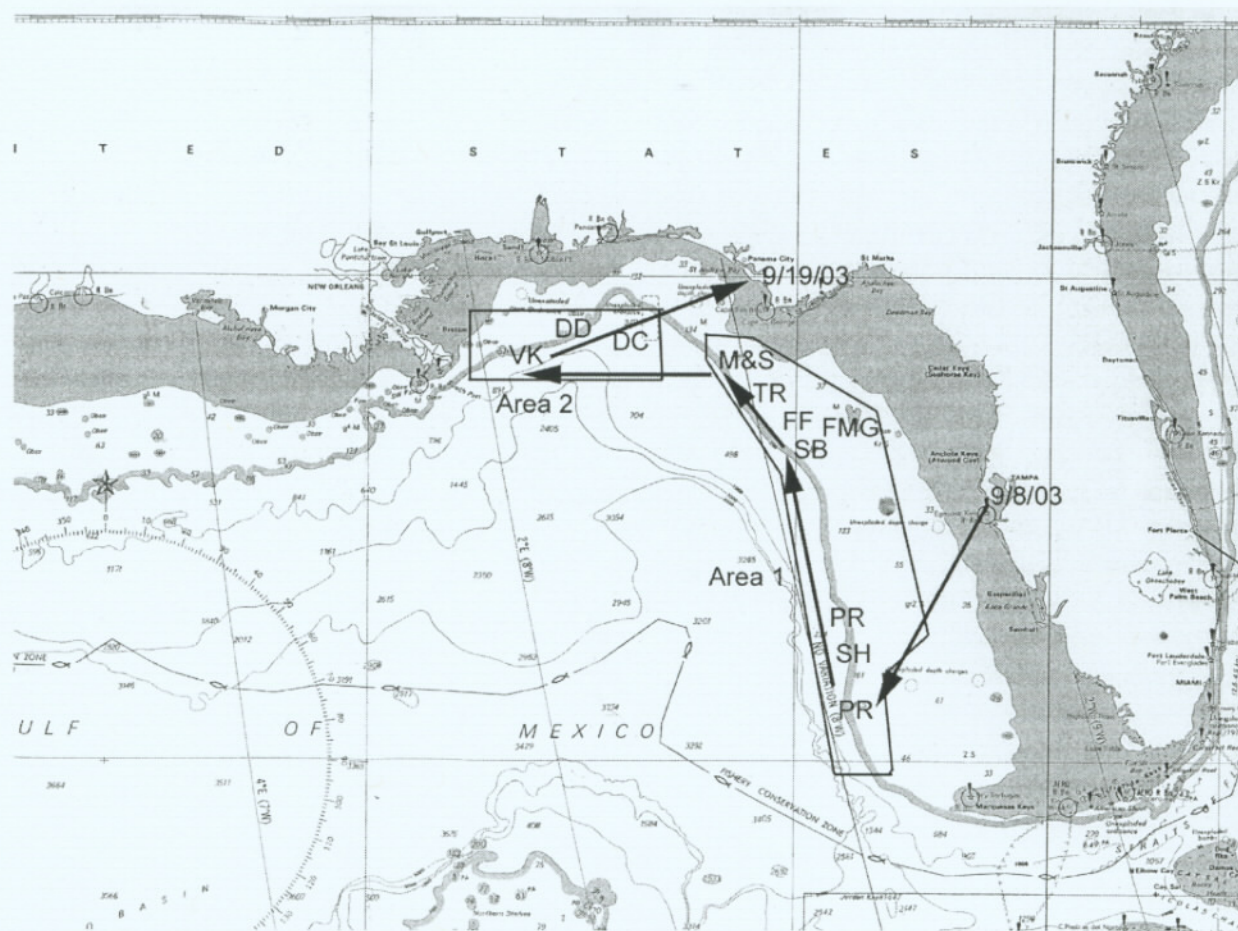
Our primary focus will be to explore and document the biodiversity of benthic, live bottom habitats at various deep-water sites (methane hydrate beds, deep-water live bottom, deep-water coral reefs, cold seeps, gas and oil seeps, salt domes, brine volcanoes, canyons, and deep-water sinkholes) in the Gulf of Mexico (GOM) to identify new resources with pharmaceutical potential. Based on our previous research in the GOM, Florida shelf, and Western Atlantic and our contacts through MMS, USGS, Florida State University, USF, Florida Marine Research Institute, Dauphin Island Marine Lab, MBARI, Syracuse University, UNC, Penn State, and others, we have developed a database of 117 potential deep water, live bottom sites within this region. In addition over 4000 oil and gas platforms are found in the northern Gulf of Mexico.

These have provided an artificial habitat for many species of invertebrates and fish. We will try to dive on some of these rigs to study the biofouling community.

The primary target sites to be explored (Fig. 1) will be:

Gulf of Mexico, continental shelf and slope off Florida, Georgia, and Alabama (~25° to 29°N and ~84° to 88°W; depth 100-3000 ft): deep-water *Lophelia* coral reefs, hard bottom, and live bottom rock reefs, seeps, sinkholes, and oil rigs. This cruise will select sites from the following Areas:

- Area 1: Region of Pulley Ridge, Florida Middle Grounds, Steamboat Lumps MPA, Madison and Swanson MPA, Twin Ridges, Forty Fathom Ledge, etc.; paleolithic limestone ledges, hard bottom reefs, sinkhole, and deep-water *Lophelia* reefs and seeps; 100-3000 feet.
- Area 2: Region of Viosca Knoll, the Pinnacles, Destin Dome, Desoto Canyon, etc. Deep-water *Lophelia* reefs, hard bottom reefs, and oil rigs; 200- 2000 feet.



VI. SAMPLING PROTOCOLS AND PROCEDURES

Collections: Samples (primarily sponges and octocorals) were collected with the SONSUB *Innovator* Remotely Operated Vehicle (ROV). The *Innovator* is equipped with a 5-function and a 7-function manipulator arms with jaws. Prior to the mission, Chief Scientist John Reed and

John McDonnough of NOAA OE met with SONSUB to discuss equipment requirements. Reed provided a video and powerpoint presentation showing what equipment is needed for a successful scientific mission with an ROV. The following equipment "wish list" was provided to SONSUB:

Basic tool and camera requirements:

- Color video camera (digital MiniDV tape deck)- mounted on pan and tilt capable of looking straight ahead or 90 dg straight down for photo transects. Data overlay on image showing date, time, depth, temperature, and ROV heading. Capability of turning this overlay off and on during a dive.
- Digital still camera- high resolution (3 mb pixel minimum), zoom, and mounted on pan and tilt capable of looking straight ahead or 90 dg straight down for photo transects.
- Two, parallel red lasers, 10 or 15 cm apart, for scale on each video and digital still cameras, that will show up in image at distance of ~10 ft. Capability of turning lasers off and on remotely during a dive.
- Six to ten collection buckets- 2.5 to 5 gallon size, capable of holding water, with lid or some cover to keep sample from washing out. Some placed on sub and some placed on TMS would be ideal.
- Manipulator- addition of clam shell grab, approximately 8 inches wide when open.
- Manipulator- addition of jaws with fingers that overlap when closed.
- Manipulator- addition of suction hose that suctions samples into closed buckets.

Top priority tools are the following:

- 1) Hydraulic clam shell grab, similar in size and function as JSLs (~8 inch diameter, 8 inch long, and 8 inch open jaw)
- 2) 10 buckets with closeable lids (~2.5 gal each; must hold water; each bucket labeled w/ numbers 1-10; 5 mounted on ROV and 5 mounted on TMS)
- 3) 1 bin w/ hydraulic lid (~3.5 ft long, 16 inches wide, 16 inches deep- capable of holding three 5-gal buckets; mounted on TMS)

Secondary priority tools are:

- 1) Manipulator jaws with interlocking fingers (similar in size and function as JSLs; ~8 inch open jaw)
- 2) Suction hose with capability of suctioning to individual buckets

With limited funding for tool design and fabrication from NOAA OE, SONSUB was able to provide the following tools for the mission:

- 1) 3 chip color camera and high resolution digital still camera- these were not operational from September 8- 14; they were returned to Houston for repair and sent via a charter boat on September 14. Data overlay was present in off/on mode on videotape. Videocamera had parallel 10 cm lasers for scale. One of the lasers malfunctioned later during the mission.
- 2) Suction sampler
- 3) Clam shell grab
- 4) Large 3' portable collection basket
- 5) 12 collection bins on TMS.

Depending on the dive site and transit requirements, 1-3 ROV dives were made each day (0800 to ~2200). During night, either SEABEAM map transects were conducted or transits were made to new dive locations. Once samples were brought on deck after the dive, they were photographed and subsampled for a variety of analytical procedures: taxonomic identification, microbial isolation, DNA/RNA preservation, chemical extraction and evaluation, antimicrobial bioassays, and invertebrate cell culture. Procedures for each are described below.

Photography: Samples were photographed *in-situ* using a modified Nikon CoolPix 990 camera. From Sept. 8-14, the digital camera was not operational, and digital still images were grabbed from the 2 chip color videocamera, which provided relatively low resolution images. Color videotapes (Mini-DV) were recorded with the ROV's pan and tilt videocamera. From Sept. 8-14, a 2 chip color videocamera was used while the 3 chip camera was in repair. Each sample was photographed in the ship's laboratory with a ruler and sample number against a gray background with a Nikon Coolpix 990 digital camera system. Digital images, and videotapes are archived in the photographic library at HBOI's DBMR. Duplicate copies are archived at NOAA OE.

Taxonomic Specimens: Taxonomic voucher specimens were prepared from each sample and stored in 20-dram scintillation vials for small vouchers and in 2-16 oz glass jars for large vouchers. Most specimens were preserved in 70% ethanol; some were fixed in formalin. Museum specimens are archived in DBMR's reference museum at HBOI.

Taxonomic Identification: Preliminary field identifications were made on board the ship. Some specimens may be shipped to other taxonomic specialists for verification. Microscopic slide mounts were made when necessary for identifications of sponges and octocorals.

Molecular Biology: A "molecular ark", or repository of frozen samples for subsequent molecular research, was initiated on this expedition and will be archived at HBOI's DBMR. For each sample, 4 subsamples (two with and two without RNA-later®) were prepared and stored at -80°C for subsequent DNA extraction.

Microbial Isolation Protocols: Samples to be used for microbial isolation were immediately subsampled after collection and prepared for plating by surface sterilization with 70% (v/v) ethanol. A cube of tissue (approx. 1 cm³) was removed using aseptic technique and placed in 20 ml sterile artificial sea water (ASW). The sample was then ground using a Virtis homogenizer and the resulting suspension serially diluted with sterile ASW. The dilution series is used as the inoculum for a series of isolation plates with selective media (e.g., for bacteria, actinomycetes, fungi). Typically, 100 µl of the 10⁰, 10⁻³ and 10⁻⁵ dilutions are used since these have been found to bracket the range at which discrete colonies are found. Inoculated plates will incubated at ambient temperature (approx. 25°C) for between 2 and 4 weeks. After incubation, discrete colonies will be picked on to fresh plates of the isolation medium, incubated and then restreaked until the isolate is axenic. Isolates will then be transferred to either Marine Agar 2216 or Emmon's modification of YpSs. All strains are maintained as both agar slant cultures and suspensions cryopreserved at -80°C at DBMR's Marine Microbial Collection at HBOI.

Preparation of Ethanol Extracts of Macroorganisms for Bioassays: Extracts were prepared on shipboard by grinding 8-10 g of the fresh organism with 20 ml of "100 %" ethanol (Pharmco). The extracts were steeped a minimum of overnight and then filtered. The concentration of dissolved solids in 1 ml of extract was determined by distilling the solvent off under a heat lamp and measuring the weight of the residue. The average concentration of the extracts is 9 mg/ml. Dependent upon the assay to be conducted, extracts may be dried down and reconstituted at 10 mg/ml in ethanol prior to assay. Extracts are stored at -25 °C at HBOI's DBMR until used.

Shipboard Chemical Profiling: To provide a preliminary indication of chemical diversity, macroorganisms were analyzed on the ship by high performance liquid chromatography (HPLC). Extracts were prepared by macerating a small piece of each specimen in ethyl acetate:ethanol (9:1 v/v). After steeping for ~12 hours, the extract was filtered, the solvent removed under a stream of nitrogen and the sample solubilized in methanol prior to analysis. Each sample was analyzed by HPLC using a reverse phase column, eluted with a linear gradient of CH₃CN in H₂O. Compounds were detected by photodiode array detection over the wavelength range 210-600 nm.

Antimicrobial Assays: To obtain a preliminary shipboard assessment of potential bioactivity of the samples, antimicrobial disk diffusion assays were conducted. Three filter paper disks (6.5 mm diameter) were dipped in each extract. After air-drying, the disks were applied to agar plates seeded with either *Bacillus subtilis*, *Pseudomonas aeruginosa*, or *Candida albicans*. Disks with antibiotics were applied to each plate for controls. After incubation overnight at 37°C, each plate was examined and zones of inhibition of growth around the disks were measured.

Invertebrate Cell Culture: Selected deep-water sponge samples were dissociated on shipboard into cell suspensions and separated by cell type using density gradient centrifugation. Cell fractions were used to initiate primary cultures at sea, and were also cryopreserved in DMSO at -80°C for subsequent research at HBOI's DBMR.

Site and Sample Documentation and Database Management: All data regarding sites and sample properties were entered into a proprietary database (Microsoft ACCESS). Each site and sample were assigned a unique number according to our existing scheme which indicates, date, site and specimen number of the collection. Collection site descriptions, including latitude, longitude, habitat, depth, temperature, salinity, current, and weather conditions; along with sample descriptions, including morphology, color, abundance, taxonomy, and photographic reference for each sample were recorded in a field notebook. All data were then entered on shipboard into the database. All underwater videotapes, and digital images of habitats and benthic species are archived in DBMR's photographic library at HBOI. A second set is archived with NOAA OE. Each videotape was documented with real-time annotations in an Excel spreadsheet which detailed habitat descriptions, fish and invertebrate identifications, sample collections, time, depth, latitude, longitude, digital still photo number, and notation of "best" video and photographs. The Excel videolog spreadsheet was provided to NOAA OE.

VII. APPLICATION TO MANAGEMENT

These were the first dives ever made to document the habitat and benthic biodiversity on the relatively unknown *Lophelia* coral reefs in the eastern Gulf of Mexico, SW Florida shelf slope. The resource potential is unknown in terms of potential fisheries, and novel compounds yet to be discovered that may produce pharmaceutical drugs. Although no plans are in the works to designate these as MPAs, they are an incredibly diverse and irreplaceable resource. Many of these structures may be ten's of thousands years old as evidence from previous carbon dating of *Lophelia* coral debris. The coral itself is slow growing, only 10-20 mm/year, and is certainly fragile. Any activities involving bottom dredging, trawling, pipeline laying, or oil/gas production could negatively impact these reefs.

OTHER DEEP-WATER MARINE PROTECTED AREAS

The Madison and Swanson Marine Protected Area was surveyed with SEABEAM map and ROV dives. Fish, invertebrate, and habitat documentation included videotapes, digital still images, Excel videolog spreadsheet, and invertebrate collections.

VIII. MARINE EDUCATION ACTIVITIES

Education Benefits- NOAA's National Education Coordinator, HBOI's media lab videographer, and a high school teacher-at-sea provided a strong educational component to the mission, including lesson plan development for grades 6-12, daily dispatches and interviews, and post cruise presentations.

IX. OUTREACH ACTIVITIES

Media Coverage- Pre- and post-cruise media events hosted regional TV and newspaper releases. Digital video highlights tape will be distributed to national markets. Outreach activities in addition to web and education included various media events and publications including: 1) Port call, St. Petersburg, 9/8/03- dockside media event, live tv and interviews; 2) *Naples News*- article, 9/28/03; 3) National Public Radio- 'The Florida Environment', Interview, J. Reed, 9/5/03; 4) National Public Radio- Jill Roberts, Interview, J. Reed, 9/5/03; 5) NASA Oceanography Outreach and OceanAge Project- Andrea McCurdy film interview with J. Reed and S. Pomponi for NOAAOE web site, 9/3/03; 6) *Washington Letter of Oceanography*, Vol. 37 (19)- article, 9/15/03; 7) Environmental News Network (ENN)- article, 9/5/03; 8) *St. Petersburg Times*- article, 9/5/03; 9) *Ascribe*, The Public Interest Newsletter- article, 9/5/03.

X. WEBSITE ACTIVITIES

NOAA's Ocean Explorer website documented real-time postings of essays, interviews, photographs, and video, from mission scientists, crew and educators, describing daily dive activities and discoveries. HBOI personnel and 'At Sea Educator' provided the NOAA OE Web site with 10 essays, 12 daily logs, 7 biosketches, and 12 factoids.

XI. SCIENCE PERSONNEL LIST

A total of 24 science, NOAA, and ROV personnel, including: 10 from the Division of Biomedical Marine Research (DBMR) Harbor Branch Oceanographic Institution (HBOI), 1 teacher at sea, 5 representatives from NOAA OE, and 8 representatives from SONSUB ROV.

Dr. Amy Wright	Principal Investigator; DBMR Division Director, HBOI
Dr. Shirley Pomponi	Co-Principal Investigator, Vice President, HBOI
John Reed	Chief Scientist; Co-Principal Investigator, DBMR, HBOI
Kathleen Janda	Microbiology, DBMR, HBOI

Tara Pitts	Microbiology, DBMR, HBOI
Gail Samples	Chemistry, DBMR, HBOI
Jane Thompson	Molecular Biology, DBMR, HBOI
Dr. Alan Duckworth	Post doc, Aquaculture, DBMR, HBOI
Priscilla Winder	Chemistry, DBMR, HBOI
Angela Ledger	Research Assistant, Molecular Biology, DBMR, HBOI
Gary Wolfe	Teacher at Sea, Eau Gallie High School
Jeremy Potter	NOAA OE, Expedition Coordinator
Laura Rear	NOAA OE, Data Manager
Taconya Piper	NOAA OE, Web Coordinator
Lynn Morgan	NOAA, GIS Data Manager
John 'Corey' Allen	NOAA, Assistant to S. Rooney

XII. CONCLUSION – A LOOK TOWARD THE FUTURE

This mission documented deep-water reefs and live-bottom habitats within US waters. Although previous geological studies have surveyed some of these sites, virtually nothing is known about the biology and biodiversity of some of these diverse habitats. Funding for ship and submersible time from NOAA's Ocean Exploration program enabled us to make 22 ROV dives, and some in areas never dived before. We were able to document the benthic macrofauna and habitats with approximately 69 hours of videotapes, 585 underwater and 255 shipboard digital still images, and 155 specimens of sponges, octocorals, scleractinian corals, crustaceans, echinoderms, and environmental samples. Initial taxonomic analyses indicate that some sponges and gorgonians may be species new to science, and some may be range extensions. Further work in collaboration with specialists will be conducted to verify these preliminary determinations. The work toward drug discovery is just beginning. Each sample was extracted and tested immediately for its natural product profile using HPLC. These initial results indicate that many specimens show promising chemistry. Continued chemical investigation will take place with funding from HBOI and discreet industrial partners as well as under an NIH funded National Cooperative Drug Discovery Group (NCDDG) grant and a new National Cancer Institute (NCI) grant on pancreatic cancer. Each sample was also tested for antimicrobial activity using disk diffusion assays conducted on board the ship. A number of active specimens were identified and will be the subject of continued investigation in the HBOI laboratories. Primary microbial culture plates prepared on shipboard will be used to obtain axenic microbial isolates. These isolates will be fermented and studied under the NCDDG project mentioned above; an NIH funded Project Program Grant which focuses on drug discovery from marine actinomycetes and combinatorial biology (PO1 CA83155); an NSF funded Biotic Inventory and Survey grant (DEB-0103668) as well as in collaboration with partners in industry. Subsamples made from each sample were also frozen and archived for the DBMR Molecular Genome ARK which will provide material for investigation by future generations. This is a new initiative at HBOI. The larger frozen samples will provide material which can be used in future bioassays and analyses.

The samples collected are certain to contain novel compounds that will show promising biological activities. These compounds will start us on the oftentimes arduous road toward new drug approval. Funding for these research missions is essential in allowing for the exploration, discovery, understanding and preservation of new habitats which contain resources that have the potential to help mankind through the discovery of new therapeutic agents which can treat existing and newly emerging diseases.

XIII. APPENDICES

[03 NOAA Cruise ROV Dive Log Notes Final.xls] :

This Excel spreadsheet with realtime annotation of each videotape (69 total) was submitted to NOAA OE on September 19, 2003 and includes the following:

detailed habitat descriptions, fish and invertebrate identifications, sample collections, time, depth, latitude, longitude, digital still photo number, and notation of "best" video and photographs.

Encouraged Preproposals of Interest to HSD

005	067
006	069
012	071
016	073
021	074
023	076
024	077
025	080
027	088
031	092
036	097
041	098
043	105
045	106
048	122
050	125
051	130
053	135
056	136
059	140
060	143
064	147
066	