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Bioluminescence and Vision on the Deep-Seafloor

July 14 – July 27

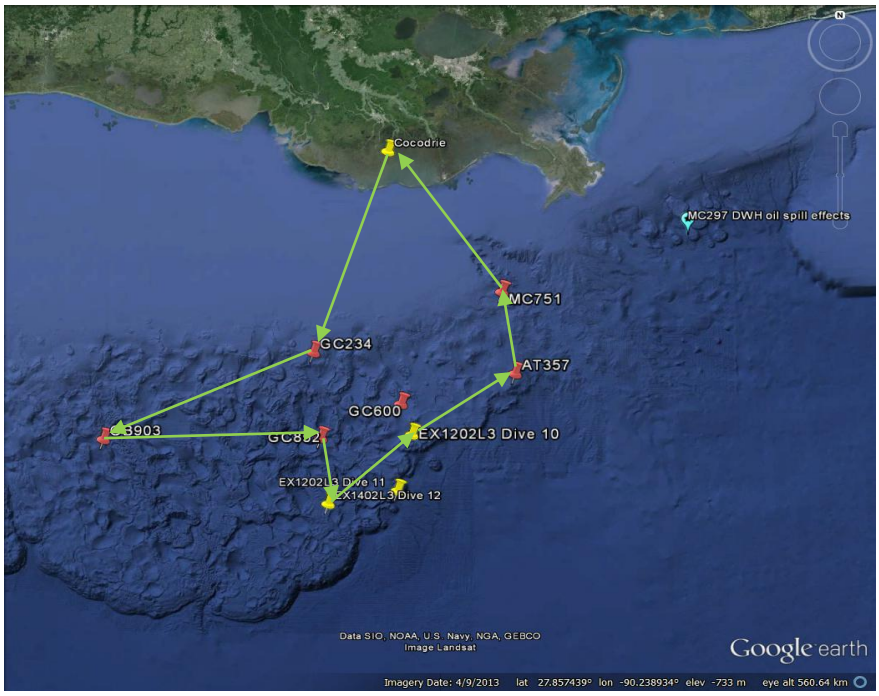
Table of Contents

Cruise Overview

a) Chief Scientist: Dr. Tamara Frank
Halmos College of Natural Sciences and Oceanography
Nova Southeastern University
8000 N Ocean Drive
Dania Beach, FL 33004
tfrank1@nova.edu
954-262-3637

b) Vessel: RV Pelican, LUMCON, Cruise number: TBD

c) Study Areas:



1) GC234: 27.74637 -91.22292; 450 – 550 m – giant seep site with corals, bacterial mats, hydrates, tubeworms

2) GB903: 27.07993 -92.81657; 1050 nm depth – lush, rich coral site discovered last year by Eric Cordes; only one previous visit

3) GC852: 27.110242 -91.166113; 1400 m depth – large seep site with big coral mount in center; lots of Bathynomus described in area

4) EX1402L3 Dive 12: 26.618528 -91.109674; 1920 m depth – briefly explorer in 2014 by Okeanos Explorer. No collections. Scattered black areas suggestive of bacterial mats. Sea pens, shrimp, polychaetes, squat lobsters, octocorals and bamboo corals also mentioned as present. Near old shipwreck

5) EX1202L3 Dive 10: 27.13613 -90.482398; 1165 m depth – briefly explored by Okeanos Explorer in 2012. Red cnidarians with crabs amongst them (looks like lithodid with eyeglow), tripod fish, some muddy bottom with what looks like Plesiopenaeus, red and white striped galatheids, scallops, Heterocarpus, parapandalus and Eugonatanotus, variety of gelatinous organisms (anemones, hydroids), some crinoids. No collections.

6) GC600: 27.36399 -90.56338; huge seeps, tubeworms, bacterial mats, mussels; 1215 m depth

7) AC357: 27.5865.1955 -89.704266; 1057 m depth; extremely lush soft coral site; seeps in vicinity as well

8) MC751: 28.194005 -89.798388; 441 m depth – large shallow site, very lush, high biodiversity

Goals and Objectives

- Explore various regions to search for new habitats, species and communities of organisms
- Search for bioluminescence on the deep seafloor
- Explore the relationship between vision and bioluminescence in benthic animals
- Explore for further examples of unusual benthic/pelagic coupling involving bioluminescence

Description of operations:

We will use the Deep-sea Systems *ROV Global Explorer* for imaging and collections. The 7-function manipulator arm will be used to gently collect organisms and place them in a BioBox. The Global Explorer has adapted two light tight, thermally insulated BioBoxes belonging to the science party, to fit on their sampling tray, next to a larger thermally insulated PVC BioBox. Thermal insulation on the trip to the surface will ensure that animals will be in excellent condition for laboratory experiments. All animals collected under white light will be photographed before the collection, as well as mechanically manipulated to test for bioluminescence. Special collection techniques will be used for crustaceans collected for vision studies. The suction sampler has been adapted with a screen insert, so that the animal will be held against the screen as the sampler is maneuvered over a light-tight BioBox. Once the suction is stopped, the animal will drop into a special collection bucket inside the BioBox, fitted with a diaphragm that will close when the suction sampler is backed out. This will ensure that the highly mobile crabs and shrimp will not be able to escape from the BioBox when the lid is open. These collections will also be conducted under two lights fitted with red filters provided by the science party. Photo imaging for community analysis will be conducted with the 24-megapixel digital photo camera fitted with 4 laser lights for size scaling. Transects will be conducted with a 10X zoom HDTV video camera which can take broadcast quality color video from 14 inches to infinity. For video imaging of *in situ* bioluminescence, an Ikegami low light HDTV camera will be used. When animals are collected or images/video are

recorded, the data will be logged on a datasheet in real time. The ROV pilots work 12 hour days, so length and number of dives/day will be determined on a daily basis depending on the site. The multibeam imaging sonar on the *Global Explorer* will also be used to detect mounds up to 300 m away.

Quantitative video transects to characterize benthic assemblages will be carried out by maintaining a relatively constant speed over the bottom while keeping the video camera as close to perpendicular to the substrate as possible. Lasers projected onto the substrate will provide scale, permitting calculation of frame area. Start and end points of each transect will be recorded and their locations used for geo-referencing. The camera will also record and display continuous date, time, depth, temperature and salinity readings.

The Medusa lander will be deployed after ROV operations have ceased for the day, and collected 24 hours later, after the end of the following day's ROV operations. It will be deployed over the side, and will return to the surface via an acoustic release system. It will provide extremely rare footage of deep-sea animals in their natural habitat, undisturbed by the bright lights or noise of the ROV, using red light illumination and an intensified camera to image animals under this dim illumination as well as any bioluminescence they may produce. Animals will be lured to the Medusa via a bait box on the end of a bar mounted directly in front of the camera, as well as an optical lure simulating a bioluminescent burglar alarm. These lures have proven very effective on previous cruise to attract large mobile predators never seen with the ROV.

Scientific sample processing:

Bioluminescence

Animals collected under white light will be removed from the BioBox, placed in chilled seawater, and brought into a light-tight lab to test in darkness for bioluminescence, using gentle mechanical stimulation and/or KCl. Spectral measurements of bioluminescence will be taken with a QE65000-Fl spectrometer, coupled to a fiber optical cable. True color images of bioluminescence will be taken with a Nikon D700 camera, pushed to an ISO of 12,800. After measurements for bioluminescence, the animals will be photographed, a piece will be stored in RNA-later, and the rest will be stored in 10% buffered formalin. On the spot taxonomic identifications will be made, if possible, and remaining identifications will be made back in land-based laboratories

Vision

Crustaceans collected under red light will be removed from the BioBox under a shroud, placed in a light-tight container of chilled seawater, and stored in chilled seawater for a minimum of 12 hours before electrophysiological experiments begin. Electrophysiological experiments will be conducted on live animals, to determine if they possess multiple visual pigments, and temporal resolution experiments will determine how sensitive the eye are to extended light sources. Eyes will also be fixed for histological studies of their structure, another indication of their sensitivity to distant light

sources. While crustaceans exposed to light cannot be used for physiological studies, they can be used for molecular studies to characterize their visual genes. Eyes collected this study will be frozen in RNAlater and RNA will be extracted once back on land. All animals used in these studies will be fixed in 10% formaldehyde after all needed tissue has been excised, to allow for verification of taxonomic identifications.

Transect data

Transect frames will be extracted from videotapes using Windows Movie Maker software, saved as high quality AVI format, and labeled with dive and transect number. Coral Point Count with Excel extensions (CPCe) version 3.4 software (Kohler and Gill 2006) will be used to calculate frame area and analyze percent cover of substrate categories (e.g., living coral, continuous hardbottom, rubble, sediment). Mobile megafauna (e.g., crustaceans, fish) will be quantified from counts from video recordings. Organisms will be photographed *in situ*, then collected, and after use in bioluminescence and vision experiments, cataloged according to museum standards. As many species as possible will be identified on site; others will be distributed to recognized taxonomic experts for identification and ultimate deposit in major museum collections.

Data for all collected samples will be incorporated into an Excel database containing: sample number, taxonomic classification, dive number, Lat/Long, collection depth, time/date, habitat type, temperature, salinity, image/video numbers, fixative, and final distribution (PI, Museum).

Outreach and Education

We are a NOAA-OE signature cruise, and have written essays and provided images for the NOAA-OE webpage. In addition, working with the WhaleTimes, Inc. Creep into the Deep Program (http://whaletimes.org/?page_id=86), we have 11 summer camps from museums across the country participating on the cruise as virtual explorers, receiving curriculum designed to go along with the research on the cruise, having the option to "ask an explorer" during the cruise, and receiving "Postcards from the Deep" during the cruise. WhaleTimes will also rewrite daily blogs into "kidspeak", as this program is designed for K-6 school age students. Museums participating include:

1. Bell Museum-University of Minnesota
2. Cincinnati Museum of Natural History
3. Florida Aquarium
4. Museum of Natural History-University of Colorado
5. Navarre Beach Marine Science Station (Florida)
6. Oregon Coast Aquarium
7. Patricia and Phillip Frost Museum of Science (Ohio)
8. Tallahassee Museum
9. University of Michigan Museum of Natural History

In addition, the GirlSTEM program, from the Kankakee School District, will also be participating. Pi Frank has complete two Skype Interviews – one with campers from the Cincinnati Museum on June 22, and one with the Tallahassee Museum on July 1st. Ruth Musgrave, the director of WhaleTimes, Inc. will be Skyping with the Tallahassee Museums again on July 22 and the Cincinnati Museum on the 23rd, while we're on the cruise, and PI Johnsen will be going a Skype interview with campers at the Colorado

Museum on August 7th. WhaleTimes, Inc. has also set up a collaboration with Marsh Myers at the Oregon Coast Aquarium, who will be featuring our cruise on their OceanScape page (http://oceanscape.aquarium.org/explore/general_articles/creep-into-the-deep-2015) and has put together a video with bios of all the PIs on the cruise on the OceanScape viddler page (http://www.viddler.com/v/6ff1e28b). PI Frank contributed slides and commentary to the powerpoint presentation for educators, headed by NOAA-OE Education Programs Manager Susan Haynes, that will be presented via a "GoTo" meeting on July 9th.
During the cruises, all members of the science party will be responsible for writing a blog and providing images, commentary and video to cruise web coordinator James Rawsthorne.

Itinerary

Staging
July 13-14: Mobilization of *ROV Global Explorer* in Cocodrie, LA, the home port of the Pelican
July 14: Arrival of science party; departure planned for 22:00 if the ROV is ready to go
July 15: Transit to Site 1; ROV deployment if time allows; Medusa deployment after recovery of ROV
July 16-25: exploration, imaging and collections with Global Explorer ROV/ Medusa deployments and recoveries; waypoints are on map; timing will depend on what we fine at each location
July 26: Transit to Cocodrie, LA
July 27th: demobilization

Personnel and Berthing Plan

Dr. Tamara Frank, Chief Scientist, Nova Southeastern University – Stateroom 7
Dr. Edith Widder, Co-PI, ORCA – Stateroom 7
Dr. Sonke Johnsen, Co-PI, Duke University – Stateroom 3
Dr. Charles Messing, Co-PI, Nova Southeastern University – Stateroom 3
Dr. Heather Bracken-Grissom, Co-PI, Florida International University – Stateroom 4
Brenna Hayes, graduate student, NSU – Stateroom 4
Katie Thomas, graduate student, Duke – Stateroom 4
Mackey Violich, ORCA, technician – Stateroom 4
Eric Burdett, graduate student, NSU – Stateroom 6
Jorge Perez Moreno, graduate student, FIU – Stateroom 6
James Rawsthorne, NOAA-OE web master – Stateroom 6
Deep-sea Systems ROV pilot – Stateroom 6
Tony Saucier, Deep-sea Systems lead pilot – Stateroom 2
Deep-sea Systems ROV pilot – Stateroom 2

Organizational Structure

Dr. Tamara Frank – chief scientist – in charge of coordinating activities on cruise; will ensure that web coordination has sufficient material to post on a daily basis; will oversee graduate students to ensure that all collections are properly notated and catalogued; conduct electrophysiological experiments

Dr. Edith Widder, Co-PI – in charge of Medusa operations; will high resolution images of animal collected for posting on the web; will test collected animals for bioluminescence potential

Dr. Sonke Johnsen, Co-PI – in charge of bioluminescence spectral analyses; will take high resolution images of animal collected for posting on the web; will take real color images of bioluminescence

Dr. Charles Messing, Co-PI – in charge of coordinating transects and identification of animals on transects; will help ensure that collected animals are properly notated and catalogued

Dr. Heather-Bracken Grissom – in charge of molecular experiments; will ensure that voucher specimens are properly stored and catalogued
Graduate students Brenna Hayes, Eric Burdett and Katie Thomas – will aid their major professors in their research; will be trained on notating and cataloging animals from collections; will transfer datasheets into electronic excel spreadsheet after every ROV dive; will be in charge of ensuring that there is sufficient cold water available for animal maintenance after every ROV dive.

Mackey Violich – will help PI Widder in set-up, launch, recovery and data download of Medusa lander.

Deep-sea Systems Global Explorer Personnel: in charge of all ROV operations; in coordination with the captain, will determine when it is, and when it is not, safe to launch the ROV. Scientists will be in the control room to request animal collections and transects, but all launch and recovery operations will be under the control of the ROV and ship's crew.

Equipment List

Standard shipboard instrumentation – all the cranes and winches required are already onboard the vessel. The small Avon has been requested to aid with the recovery of the Medusa. The Global Explorer ROV will be provided by Deep-Sea Systems, Inc. The Medusa lander will be provided by ORCA.

Due to the size of the ROV van, there isn't space to put the refrigerated van. PI Frank has shipped four portable refrigeration units (Koolatrons) to be used for animal maintenance. Onboard refrigerator and freezer will be used to cool water down that will be used for animal maintenance. All equipment required, as described in the scientific sample processing, will be shipped to Cocodrie by the science party, including the Medusa lander.

Deposition of Data

Copies of all video tapes will be provided to James Rawsthorne, the NOAA-OE web coordinator, before we leave the ship. Copies of all data logs, handwritten as well as those input into the electronic database, will be provided, as will representative images of every specimen collected. Images to be used in publications will be accessible after the manuscripts have been published – copies will be given to NOAA-OE sooner if required with the request that they not be posted until after publication (bioluminescence images are the most likely to fall into this category.
The database with all sample data will be provided to NOAA-OE before we leave the ship, and will be continually updated as species identifications are made/verified. Specimens will initially be shipped Nova Southeastern University and then distribution accordingly. Specimens collected for visual physiology will be housed in TM Frank's climate-controlled laboratory at NSU. All material for molecular work will be preserved in RNAlater and stored at -80C. All eyes will be associated with whole specimens that are cataloged in a digital database. The database currently in place at FIU (FileMakerPro) allows for the following data entries: full taxonomic classification, catalog number, photo voucher/digitized images, associated locality information with an interactive map, geographical

distribution, fixative, collector field notes, information about associated organisms and a remarks section. The database also includes a molecular component that can be used to track the entire genetic history of the organism; from extraction to sequence data. All genetic data can be linked back to a museum-vouchered specimen. All vouchers, specimens and tissues will ultimately be archived in the Florida International University Zoological Collection (HBG), National Museum of Natural History (USNM), or other appropriate repositories. Bioluminescent specimens will be housed in S Johnsen's climate-controlled laboratory at Duke University. Rare or unusual species will be provided to the National Museum of Natural History, Smithsonian Institution, to add to their collections. Unidentified specimens will be sent to professional taxonomists for identification. Specimens other than those utilized for bioluminescence or vision studies will be housed in CG Messing's climate-controlled laboratory at the NSU Oceanographic Center pending identification in-house or distribution to professional taxonomists for identification, in advance of deposition in the permanent collections of the National Museum of Natural History, Smithsonian Institution, Washington DC. Duplicate materials may be distributed to permanent collections at other recognized institutions associated with the identifying taxonomists (e.g., Los Angeles County Museum; Natural History Museum, London; Muséum national d'Histoire naturelle, Paris).

Reports and Publications

The QuickLookReport will hopefully be provided before we leave the ship; if not, then within 10 days after the end of the cruise. The Semi-Annual Report and Final Report will be generated before the required deadlines. Data collected for bioluminescence and vision studies/habitat characterizations/new species will be published in peer-reviewed journals and presented at national conferences by the respective PI or taxonomic expert.

Emergency Information

Every member of the science party has filled out the "medical forms" provided by the RV Pelican, which requires emergency contact information. As this form contains personal medical information, I sent them to every member of the science party with the request that they be sent directly to Nic Allen of LUMCON, rather than to me.

Communications

In addition to internet access, the *RV Pelican* has 2 standard horizon quantum VHF marine radios, an ICOM HF Marine IC-M802 single side band marine radio in the wheelhouse, multiple handheld VHFs, a cellular phone signal booster allowing signal propagation throughout the ship, an iridium satellite phone, and a fleet broadband satellite phone. In case of emergency, the *R/V Pelican* can be reached directly or via the LUMCON Marine Center during normal working hours. LUMCON Marine Center phone number is (985) 851-2800. The ship can be also be reached via satellite connections at 985-746-4369 on kvh and 985-635-0991 on iridium.

Hazmat Inventory

Chemical (MSDS sheets attached in appendix) that will be used during the cruise are:

10% formalin (formaldehyde spill kit will be in science lab)
70% ethanol
RNA-later
Gluteraldehyde

Osmium tetroxide

Meals

All meals are provided by the Pelican. None of the science party has any dietary restrictions. Meals are served at the following hours:

Breakfast 0600-0700
Lunch 1200-1300
Dinner 1800-1900

If operations take place during normal meal hours, the Galley crew will be alerted ahead of time.

Appendix

Detailed Project Description

Goals and Objectives

Bioluminescence is a fascinating phenomenon that is relatively rare on land but is common in all the world's oceans, with 14 animal phyla (so far) having marine bioluminescent representatives (Herring 1987, Haddock *et al*. 2010). Although bioluminescence has been extensively studied in the pelagic zone (water column), most information on its occurrence in the deep-sea (>200 m) benthos is fragmentary and anecdotal, a result of the difficulty in retrieving intact benthic organisms from trawls and dredges. However, on a 2009 OE-funded cruise to the Bahamas (Biolum 2009), using temperature and light-insulated collecting containers on the *Johnson-Sea-Link* submersible, we collected numerous species of cnidarians, echinoderms, crustaceans, cephalopods, sponges and one species of annelid worm alive and in excellent condition from 3 sites (Frank *et al*. 2012, Johnsen *et al*. 2012*,* Messing submitted). During this expedition, we obtained the first true color images of bioluminescence for many deep-sea benthic species. In addition, we discovered the first bioluminescent species of anemone.

This systematic survey, however, revealed the surprising result that, at least in this area, bioluminescence in benthic species was far less common than bioluminescence in mesopelagic animals from similar depths, challenging the existing paradigm that bioluminescence would be as common in the benthos as in the pelagic zone at similar depths (Herring, 1983; Widder et al. 1983). Fewer than 20% of the 100 species collected were bioluminescent (Johnsen *et al.* 2012). However, this was the first systematic survey of deep-sea benthic bioluminescence to be performed, so the relative dearth of bioluminescent taxa may be location-specific and not a universal condition. Further surveys of bioluminescence in deep-sea benthic communities in the Gulf of Mexico will go a long way towards answering this question. It is important to recognize, however, that while bioluminescence was not widespread among benthic species in this location, it was nonetheless prevalent due to the dominance and size of the benthic species that were bioluminescent. In addition, the frequency with which planktonic bioluminescence was triggered due to currents carrying plankton-laden water across these benthic organisms was quite common in this structurally complex benthic habitat dominated by tall stands of arborescent anthozoans, e.g., gold coral and octocorals (Johnsen *et al.* 2012).

Although these regions had been visited many times on other expeditions (e.g., Neumann et al. 1977; Messing et al. 1990), our ability to bring specimens to the surface in pristine condition resulted in the discovery of several new taxa: a new genus of bioluminescent zoantharian gold coral (formerly *Gerardia* sp.), one new species each of homolid glass-sponge-carrying crab and primnoid octocoral, and three (possibly four) new species of Crinoidea, including a new genus.

We also discovered a potential new form of benthic/pelagic coupling, involving the use of bioluminescence within crab and cnidarian associations. Numerous species of crabs, particularly galatheoid anomurans, live around or on deep-sea cnidarians (e.g., gold, stony, soft, and black corals), and several OE-funded cruises (*Biolum 2009* in the Bahamas*; Deep-Scope 2005* in the Gulf of Mexico)

frequently found two, *Eumunida picta* and *Gastroptychus spinifer*, associated with the new gold coral genus and various black coral (Antipatharia) species.

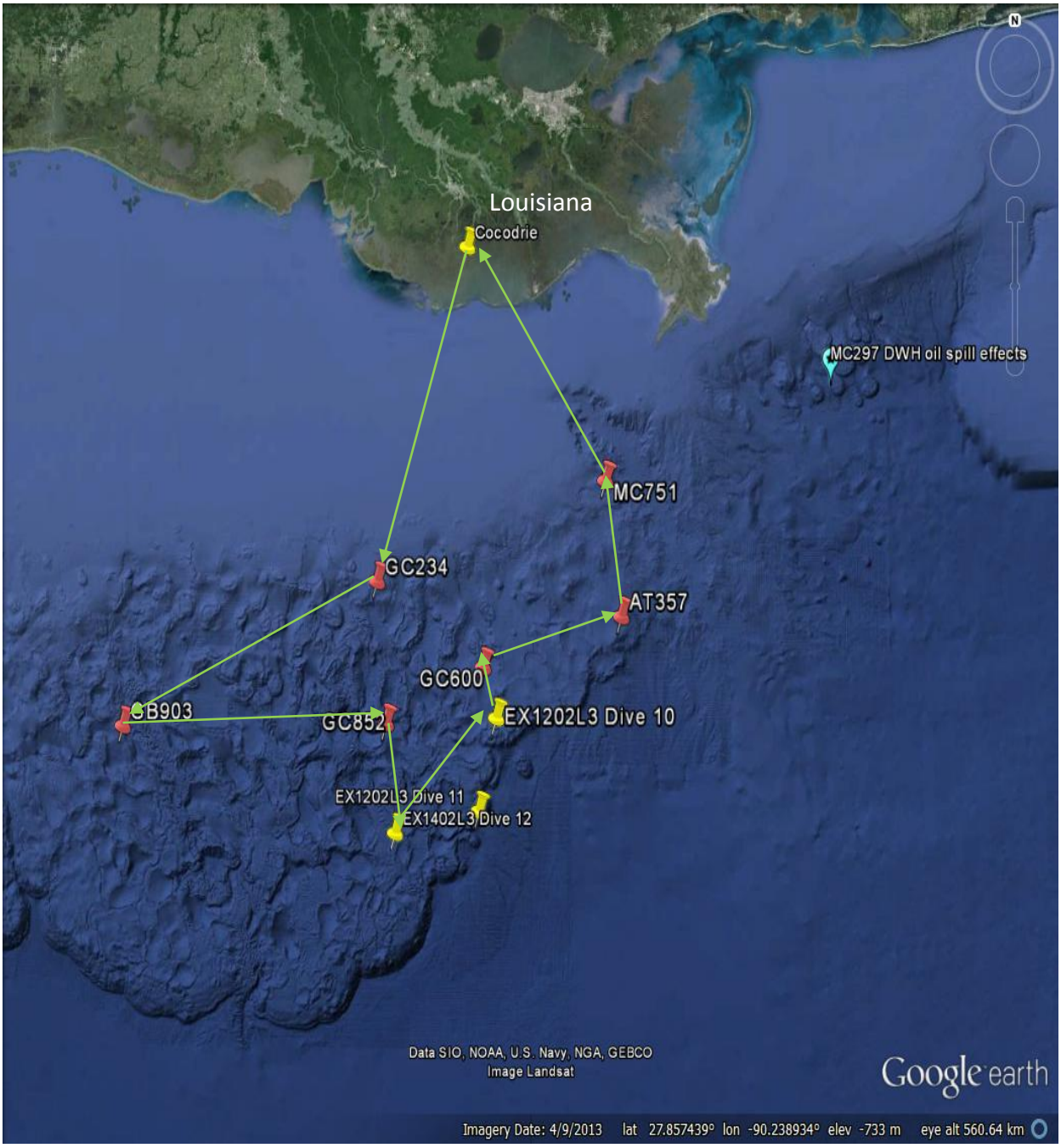
Observations under red and light white indicated that the crabs are stationary on these organisms for long periods of time, and periodically use their claws to pick particles off their hosts and bring them to their mouths. The spectral sensitivities of all seven species of deep-sea benthic crustaceans studied to date (all on our OE-funded expeditions) peak in the blue wavelengths, but those of the two species (*E. picta* and *G. spinifer)* associated with cnidarians have a second sensitivity maximum at violet/ultraviolet wavelengths, which may be an adaptation associated with bioluminescence (Frank *et al*. 2012). In those tall, sessile, aborescent anthozoans that produce it, bioluminescence is green-shifted (Johnsen *et al*. 2012) compared to most of the pelagic bioluminescence that has been measured to date (Herring 1983, Widder *et al.* 1983; Haddock and Case 1999; Widder 2010). In a remarkable 10-second exposure taken from the *JSL* during Biolum 2009, this color difference is clearly displayed, as a planktonic animal impacting a stand of gold coral gives off a much bluer bioluminescence than the greenish bioluminescence of the gold coral. This gave rise to the intriguing hypothesis that the species living amongst the stands of bioluminescent cnidarians may be "color coding" their food (this idea received major international and national press coverage – see reference list), using the two visual pigments to distinguish between the green bioluminescence of their preferred cnidarian substrate from the blue bioluminescence of their preferred pelagic food (Frank *et al.* 2012). As this is the first time that the bioluminescence/visual ecology of this cnidarian/crustacean deep-sea symbiosis has been studied, this hypothesis deserves further study. Symbioses between decapod crustaceans and cnidarians are well known in shallow water habitats. It has been hypothesized that the crustaceans benefit from this association by being protected from predators, either via camouflage or because predators avoid the unpalatable cnidarians (Wirzt & d'Udekem 2008, Duris et al. 2012), as well as by nutrition, as some species eat portions of the host cnidarian (Suzuki and Hayashi, 1977; Fautin et al. 1995, Wakabayashi et al. 2012). In these deep-sea species, the cnidarian may indirectly be providing a source of nutrition for the crustacean, either by providing a means for the crustaceans to get up into the current to catch drifting plankton, or by catching plankton on sticky tentacles that the crustaceans pick off. In addition to our observations with *E. picta* and *G. spinifer*, during the Okeanos Explorer surveys in the Gulf of Mexico at the proposed areas of operation (West Florida Escarpment and south of DeSoto Canyon), numerous images were taken of anomuran crabs and shrimp with usually broad and long chelae associated with various cnidarian taxa. These animals were not collected, so nothing is known about the crustacean visual systems or the bioluminescence of the cnidarians. These images indicate that these relatively unexplored regions of the Gulf of Mexico are prime locations in which to observe these types of symbioses, as well as discover new sources of bioluminescence.

Another novel use of bioluminescence is based on the as yet unverified idea that accumulations of marine snow and detritus on the ocean floor might contain bioluminescent bacteria (Lampitt et al. 2001). *Photobacterium* spp. occur throughout the marine environment (Ast and Dunlap 2005), and luminous bacteria are known to colonize crustacean and fish carcasses (Wada et al. 1995), and fecal pellets (Andrews et al. 1984, Zarubin 2012). Nishida et al (2002) suggested that the resulting background glow may be used as a cue by deep-sea scavengers. Our OE-supported research on the

visual physiology of the isopods *Booralana tricarinata* and juvenile *Bathynomus giganteus* collected at 600 m depth off Little San Salvador, Bahamas, supports this hypothesis. Their maximum flicker fusion frequency (the highest stimulus rate at which the eye can produce electrical responses that remain in phase with a flickering light) is 4 Hz, the lowest ever measured in a crustacean (Frank *et a*l. 2012). The flicker fusion frequency roughly reflects the eye's integration time, and can be thought of in terms of the shutter on a camera – the lower the flicker fusion frequency, the longer the shutter would remain open. The integration time of this isopod's eye is ~250 msec, far greater than the 70-105 msec measured in deep-sea benthic crabs (Frank *et al.* 2012). For comparison's sake, the integration time of a light-adapted human eye is 16 ms. This extremely long integration time indicates that this isopod probably cannot track moving prey, because moving objects, particularly those emitting luminescent flashes, would be blurred. As deep-sea isopods are thought to be scavengers (reviewed in Barradas-Ortiz *et al*. 2003), an eye with a very long integration time would operate like a camera with a very slow shutter speed, allowing the animal to see dimly glowing detritus, aiding the animal in finding a food target. However, the presence of bioluminescence on decaying mats of detritus has never been verified, and we propose to study this phenomenon during the proposed research expedition.

Using our combined expertise in bioluminescence, taxonomy, visual ecology, *in situ* imaging, and molecular biology, we propose to use the unique capabilities of the *Global Explorer* ROV and the Medusa lander to continue our exploration of the deep-sea benthic environment, identifying new communities of organisms, looking for new sources of bioluminescence and further studying crab/cnidarian associations. This ROV has superb collecting capabilities, and we will be able to use our low-light cameras on the ROV to observe and photograph stimulated bioluminescence of living organisms *in situ*. The Medusa lander is an upgrade of the Eye-in-the-Sea (EITS) system that gave us phenomenal footage of deep-sea animals in the natural settings on previous expeditions (see below) and captured the first *in situ* video of the giant squid *Architeuthis dux* (Widder 2013). The EITS was limited because it needed to be deployed from a submersible. The Medusa lander can be deployed over the side, and is designed to return to the surface using a timed/release system, substantially enhancing its capabilities. Using both a low-light camera mounted on the ROV and the low light camera on the Medusa, we will also attempt to image background bioluminescence of decaying material or bacterial mats, essentially imaging the deep-sea floor by its own light. The Medusa camera system will be deployed for 24 hour time intervals, allow us to unobtrusively examine the interactions between crustaceans and cnidarians *in situ* as well. Utilizing thermally insulated, light-tight BioBoxes on the ROV, we will be able to collect benthic specimens and bring them to the surface alive, in cold water and in the dark, enabling us to identify the organisms that produced bioluminescence *in situ*, conduct spectral measurements of their luminescence, and study their visual physiology.

Primary Operating Area Map



Data Management Plan

- Images and videos of habitat and organisms from *ROV* dives under white light
  - Copies will be made and provided to NOAA-OE either one the cruise (if we have a NOAA-OE database manager participating on the cruise) or within a month after the cruise
- Images and videos of organisms taken with low light cameras/Medusa lander/laboratory images of bioluminescence
  - Representative images will posted immediately on the expedition webpages (NOAA-OE, NSU @sea webpage, WhaleTimes Creep into the Deep) and be available for publicity purposes immediately after the expedition
  - Images to be used in publications will be accessible after manuscripts have been published – copies will be given to NOAA-OE sooner if required with the request that they not be posted until after publication
- Data for all collected samples will be incorporated into an Excel database containing: sample number, taxonomic classification, dive number, Lat/Long, collection depth, time/date, habitat type, temperature, salinity, image/video numbers, fixative, and final distribution (PI, Museum). This database will be provided to NOAA-OE within one week of the cruise, and continually updated as species identifications are made/verified.
- Specimens collected for visual physiology will be housed in TM Frank's climate-controlled laboratory at NSU. Rare or unusual species will be provided to the National Museum of Natural History, Smithsonian Institution, to add to their collections
- All material for molecular work will be preserved in RNAlater and stored at -80C. All eyes will be associated with whole specimens that are cataloged in a digital database. The database currently in place at FIU (FileMakerPro) allows for the following data entries: full taxonomic classification, catalog number, photo voucher/digitized images, associated locality information with an interactive map, geographical distribution, fixative, collector field notes, information about associated organisms and a remarks section. The database also includes a molecular component that can be used to track the entire genetic history of the organism; from extraction to sequence data. All genetic data can be linked back to a museum-vouchered specimen. All vouchers, specimens and tissues will ultimately be archived in the Florida International University Zoological Collection (HBG), National Museum of Natural History (USNM), or other appropriate repositories.
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- Data collected for bioluminescence and vision studies/habitat characterizations/new species will be published in peer-reviewed journals and presented at national conferences by the respective PI or taxonomic expert.

MSDA Sheets