National Marine Sanctuaries as Sentinel Sites for a Demonstration Marine Biodiversity Observation Network (MBON)

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Objective of Research:

To design a demonstration Marine Biodiversity Observation Network (MBON) to monitor changes in marine biodiversity within three US National Marine Sanctuaries (NMS): Florida Keys and Flower Garden Banks in the Gulf of Mexico, and Monterey Bay on the U.S. west coast.

Project funds are administered under a Cooperative Agreement structure between NASA and the University of South Florida (USF). Additional partners in the MBON project are funded via subcontract issued by the USF or by direct or in-kind funding through NOAA IOOS, NOAA NMFS, and NOAA Exploration. Under the Cooperative Agreement, the project elements managed by USF are responsible for the following agreed objectives:

1. Implement a demonstration Marine Biodiversity Observation Network (MBON) to monitor changes in marine biodiversity within the Florida Keys National Marine Sanctuary and the Monterey Bay National Marine Sanctuary.
2. Integrate, synthesize, and augment information from ongoing monitoring programs to:
   a. Provide geographically-integrated time-series metrics of biodiversity and ecosystem health;
   b. Define a minimum set of observations required for implementing a practical MBON;
   c. Develop environmental DNA technology and autonomous sample collection methods for conducting biodiversity assessments; and
   d. Bring biodiversity measurements together in a relational database with links to national and international databases.
3. Establish a protocol for rapid use of MBON information by stakeholders in the National Marine Sanctuary System and elsewhere.
4. Build understanding of the connections between marine biodiversity and the social-economic context of a region.
5. Develop a plan to transition the MBON into an operational system through partnerships with the U.S. Government and other partners.
6. Integrate this MBON activity into the international Group on Earth Observations Biodiversity Observation Network (GEO BON).
7. Prepare for, participate in, and respond appropriately to reviews of the project.
8. Participate in scientific meetings or professional society meetings identified by U.S. Government program managers as pertinent to project goals.
9. Inform the relevant U.S. Government program managers of any results nearing publication and the release of final data products so that they might prepare for the announcement and any associated publicity and/or public outreach.
10. Data produced under this cooperative agreement will be available without restriction as to its disclosure, use or duplication except as otherwise negotiated by NASA and the University of South Florida. The goal is full and open exchange of data and metadata with minimum possible cost, delay and restriction.

NASA shall be responsible for the following contributions to the joint activity:

1. Conduct regular reviews of the project awarded under this cooperative agreement.
2. When applicable, participate in science community meetings and conferences pertinent to this award.
3. Inform the Principal Investigator of any planned publicity and/or public outreach activities related to this project.
4. Assist the Principal Investigator and other project personnel in integrating this activity into GEO BON.

Our effort has focused on how to best contribute to assessments of ecosystem integrity, advancing protection of marine resources, and promoting conservation. We have started a very substantial effort to use novel eDNA techniques and ongoing observations to evaluate habitat diversity, diversity of lower to higher trophic levels, to define the ecological state variables responsible for significant change in biodiversity indices, and help identify invasive species. Multidisciplinary remote sensing is now being used to evaluate dynamic 'seascapes' to extend the spatial footprint of the in situ data.

These time series of biodiversity and environmental observations will help construct conceptual and forecast models of the inter-relations between human dimensions, climate and environmental variability, and ecosystem structure at multiple trophic levels. The program includes graduate education and public outreach efforts to be coordinated with the Sanctuary programs, IOOS, and various other regional NOAA outreach and extension programs.

Our group has spearheaded coordination efforts and leverage resources with two other MBON demonstrations to be implemented in the Channel Islands NMS and the Chukchi Sea shelf, Alaska, as well as internationally through the GEO BON framework. We have actively pursued conversations on how to transition the MBON to operations in partnership with NASA, NOAA and the IOOS program.

Project Scope and Approach:

The project will help to determine the minimum set of variables needed to operationally assess changes in characteristic biodiversity and to regularly update Sanctuary 'Condition Reports'. We are reviewing ongoing observation programs, strategically adding key observations, and developing data synthesis efforts in coordination with the IOOS.

Our approach addresses the following questions:

a. What are the links between climate and biodiversity indices developed for different trophic levels within the Sanctuaries, and what are their baselines and time and space scales?
b. What physical processes affect the Sanctuaries at these characteristic scales?
c. What are the roles of mesoscale and basin-scale dynamics in forcing ecosystem variability?
d. How does physical forcing regulate ecosystem dynamics (community and population structure, trophic energy transfer) at seasonal, interannual and decadal time-scales?
e. Can novel genetic techniques be used to synoptically assess the characteristic biodiversity within Sanctuaries, and if so how are these transitioned to operational entities?

To answer these questions the MBON started developing the following tasks in Y1:

1. **Integrate and synthesize information from ongoing biodiversity monitoring programs within an established infrastructure:** We build on existing infrastructure and programs that currently assess marine biodiversity within NOAA's National Marine Sanctuaries system, working with the IOOS RAs in central data management and outreach roles. We seek to establish baseline information on the characteristic ecosystems, evaluate the spatial extent and timing of changes in biological communities across large coastal regions, and understand the mechanisms underlying the patterns and variations using satellite remote sensing in tandem with in situ data.

2. **Define the minimum set of biodiversity observations required for implementing a practical, useful, and sustainable MBON:** We seek to identify the minimum set of variables needed to evaluate possible changes in characteristic biodiversity and ecosystem state within the Sanctuaries. We have started gathering data from time series of observations collected by ongoing regional research programs and the IOOS RAs to identify the ecological state variables responsible for significant change in biodiversity indices, and to formulate algorithms that complement the output of physical forecast models. Assessments will integrate these three interlinked levels:
   a) *Time-varying habitat*. We are working on a set of rules that help define baseline biogeographical regions and comparisons across hierarchical 'seascapes'. We are working to merge satellite observations, surface and subsurface in situ observations, and models to classify dynamic, synoptic seascapes.
   b) *Lower trophic levels.* We are moving aggressively to develop indices of bacteria and phytoplankton diversity, bloom timing, duration, and magnitude. These indices will be evaluated for applicability to evaluate as ecosystem health and changes in higher trophic levels, in the water column as well as in benthic communities.
   c) *Intermediate and higher trophic levels.* We have started to gather the datasets needed to recognize distinct population segments of organisms that occupy a specific seascape at any particular time. This will be done following two primary strategies:
      - Links with existing biological assessments:
        - NOAA Fisheries Science Centers (Southeast, Southwest)
        - State agencies (Florida Fish and Wildlife Research Institute)
        - Sanctuary Integrated Monitoring Network (SIMoN).
        - Monterey Bay Time-Series program
      - Environmental DNA (eDNA) collections.

3. Technology development: We have initiated a comprehensive genomic program to detect the presence of a broad range of organisms (from bacteria to large marine vertebrates) using a unified analysis framework for processing environmental DNA (eDNA) across multiple trophic levels.
4. Data management: The MBON requires linking observations from multiple stakeholders that presently generate biodiversity datasets with distinct metadata, format, and data characteristics. GCOOS, CeNCOOS, Axiom Data Science, the Florida Fish and Wildlife Research Institute (FWRI), and OBIS are coordinating standardizing of metadata and biodiversity information. The MBON and the RA's are investing in an interactive data

product and visualization tool to synthesize information for management and prioritized environmental response.

5. Establish a protocol for MBON information to dynamically update Sanctuary Condition Reports: The Sanctuary Condition Reports synthesize information collected with the Office of NMS (ONMS) System-Wide Monitoring (SWiM) framework, which addresses 17 questions about the state of the Sanctuaries in water quality, habitat, living resources, and archeological legacy. The MBON will help to maintain up-to-date Condition Reports by providing a stream of data and interpreted products designed to answer these SWiM questions: 1) What is the integrity of major habitat types and how are they changing? 2) What is the status of biodiversity and how is it changing? 3) What is the status of non-indigenous species and how is it changing? 4) What is the status of keystone and foundation species and how is it changing? 5) What is the status of other focal species and how is it changing? 6) What are the levels of human activities that may influence living resource quality and how are they changing?

6. Understand the linkages between marine biodiversity and the social-economic context of a region. We have started a dialogue with the ONMS and science staff and Sanctuary Advisory Council Members at the MBNMS and FKNMS to understand the social and economic context of each Sanctuary Condition Report.
   We are starting to evaluate application of the Ocean Health Index to the Sanctuaries (see http://www.oceanhealthindex.org/).

Expected products and Outcomes:

1. Conceptual models of ecosystem structure within each Sanctuary including key indicator groups (Years 3-5).
2. A centralized data management plan:
   a) integrates existing biodiversity information spatially and over time from each Sanctuary (Years 1-2),
   b) an implementation strategy for IOOS and Sanctuary programs (Years 4-5).
3. A 'seascape' framework to integrate merged satellite and in situ observations (Years 2-5): Implementation of seascape products into IOOS Data Management and Communication (DMAC) and ERMA systems (Years 3-5).
4. Coordination of sample collection, methods, and data integration into DMAC:
   a) Design of an alert system triggered by changes in diversity indices that is readily accessible to the Sanctuary's Research Coordinators, researchers at scientific institutions and regulatory agencies, and the public in general (Years 2-3).
   b) Implementation of the framework into IOOS DMAC and ERMA (Years 4-5).
   c) List of minimum observations required for an MBON for each Sanctuary (Years 3-4).
5. Development, validation and deployment of eDNA technologies for continuously assessing biodiversity in the Sanctuaries. Identify and train appropriate personnel in the use of these technologies.
6. Design and implement an interactive online biodiversity geospatial information system (Years 2-4).
7. Implementation of a protocol for dynamic, digital input to the Sanctuary Condition Reports:

a) Include the publication of continuously updated indices of status and trends in key Sanctuary state variables (Years 3-5)

8. Evaluation of socio-economic value and policy options
   a) Workshop in Year 2
   b) Annual information needs assessments in Monterey, FL Keys, Flower Garden Banks using smaller workshops and stakeholder surveys

Progress Summary/Accomplishments:

The program effectively started in effectively in October 2014 after delays in processing grant paperwork. The MBON team immediately initiated the coordination and implementation of research, DMAC, E&O activities. There are six general components to the MBON project:

-MBON Data Management and Communications (DMAC)
-Field data collections
-Genomics/eDNA
-Seascapes and high-res satellite remote sensing products
-GEO BON MBON
-PR and E&O activities

A communication strategy to link within and across MBON *task teams* was put in place. This included the creation of 12 listservers that allow exchanging email among members of the different task teams and across all colleagues within the entire MBON. Several regional kick-off meetings and teleconferences were carried out to coordinate efforts at each Sanctuary and within disciplines (i.e. eDNA, seascapes, DMAC, E&O). Also, MBON participants gathered at the All-hands kick-off meeting in Miami (March 2-4) to: 1) design a strategy for integrating and harmonizing the various components of the project, 2) assess resources and overall capacity, as well as next steps towards achieving the goals of the MBON, 3) identify pitfalls and challenges to achieving these goals, 4) discuss collaborative approaches for developing end-products and deliverables, and 5) examine possible mechanisms for transitioning MBON to operations.

Field efforts at MBNMS and FKNMS were planned, coordinated and are being executed. Development and testing of eDNA methodology, as well as field data collection, has started at both the MBNMS and FKNMS.

Initial discussions and efforts are being led by GCOOS and CeNCOOS RA's, FWRI, and Axiom Data Science. This seeks to develop mechanisms and tools for synthesizing, integrating, and ingesting environmental and biological data in a centralized MBON DMAC system. The system will help generate products and distribute maps targeted to various end-users. These efforts include substantial interactions with OBIS and GEO BON.

A more detailed description of progress follows below.

MBON Data Management and Communications (DMAC) Team Activities:

A comprehensive data management and communication (DMAC) plan for the MBON is currently being prepared jointly by GCOOS (M. Howard and B. Kirkpatrick), CeNCOOS (D. Anderson), Axiom Data Science (R. Bochenek), and FWRI (K. O'Keife). The initial steps of the DMAC plan consist of inventorying and evaluating information from ongoing biodiversity programs operating in FKNMS and MBNMS, as well as data collected in the framework of the MBON demonstration as described below, and identifying data format standards that are compatible with national and international data repositories. The DMAC team is also working hand-in-hand with colleagues at OBIS to establish data sharing mechanisms that also link internationally via I-OBIS. We are working with GEO BON to help promote international marine biodiversity data collection and information systems. Additionally the team is looking to collaborate with REEF.org and the possibility of using their citizen science based fish survey project as an ancillary dataset to help evaluate biodiversity within FKNMS.

The FWRI team has developed a fully interactive and customizable, JavaScript based GIS online data viewer for the pilot FKNMS MBON as a pilot FKNMS DMAC system: (http://ocean.floridamarine.org/indevelopment/MBON_FKNMS/). This website hosts the majority of the data and metadata layers generated by independent programs collecting biological observations in this Sanctuary such as the Reef Visual Census species richness data. The tool allows users to assess the spatial distribution of existing data layers across the Sanctuary and plan activities for the FKNMS pilot MBON.

FWRI is constructing an ecosystem model that incorporates FKMNS wide biological data to describe changes in trophic interactions over time and space. The goal is to use the ecosystem model as one method for monitoring changes in biodiversity by focusing on the status and trends of functional groups within the ecosystem. Model construction is in the first phase: identifying data gaps, data exploration, and identifying relevant covariates. The Ecopath model has been selected as being the model most applicable to describe biodiversity in the Keys.

A MBON research workspace group has been established and configured by Axiom to assimilate data and metadata from historical records and ongoing programs at both Sanctuaries that will feed MBON. The research workspace also allows MBON collaborators across all MBON funded projects to share data and files containing information such as cruise plans, sampling protocols, data summaries, presentations, and reports. Axiom is also developing an online MBON data portal with real-time data visualization and synthesis capabilities designed to assess ecosystem state and biodiversity in the Sanctuaries (https://workspace.ioos.us/). A proof of concept portal has been deployed by Axiom to provide public access to relevant regional MBON data sets and provide capabilities for visualizing and integrating these resources (Figure 1). Axiom is already importing biological data layers from FKNMS and MBNMS into the MBON portal; for example, the portal is directly linked to FWRI's ArcGIS REST service (http://ocean.floridamarine.org).

Future capabilities will enable users to create customized queries and data distribution maps for each Sanctuary, and download GIS layers for use in desktop GIS or Google Earth. The tools will

ultimately serve as a mechanism for dynamically updating Condition Reports generated by Research Coordinators and ONMS. A prototype version of this tool will be ready in 2016.

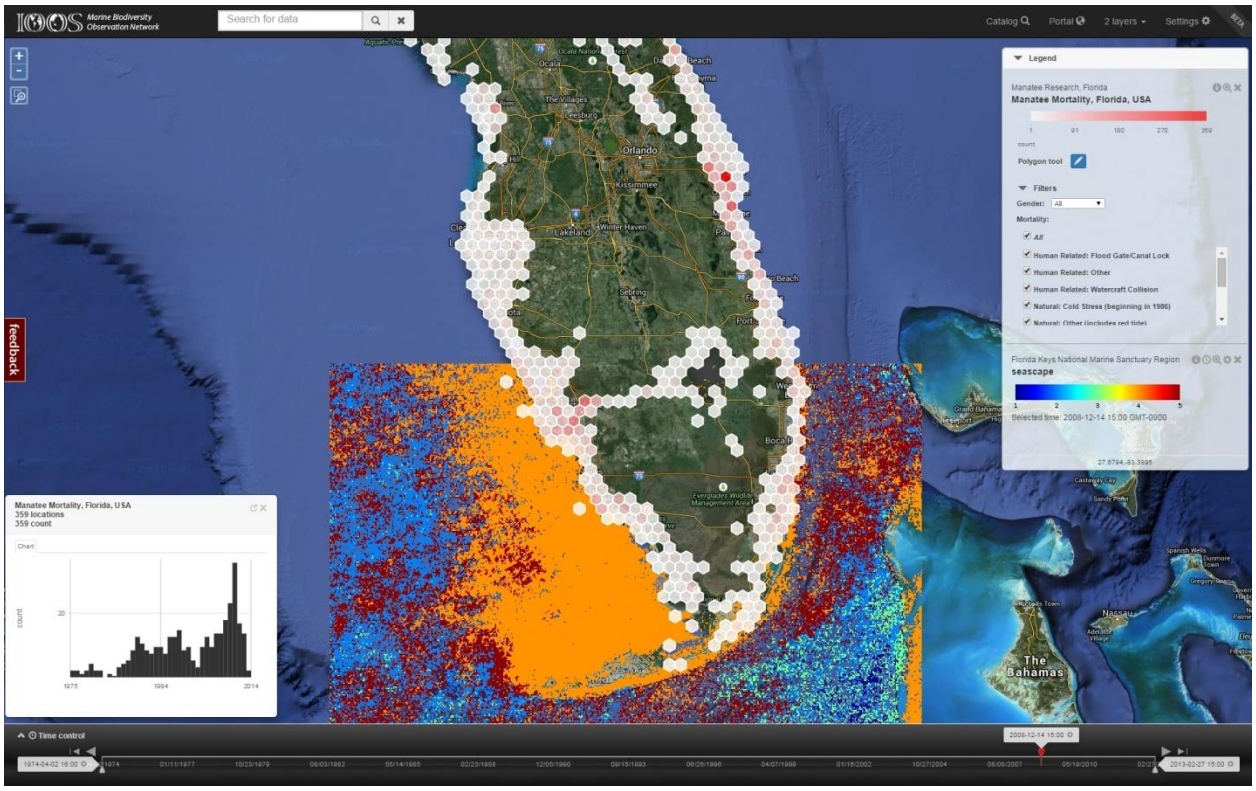

Figure 1. Screenshot of proof of concept MBON data portal integrating a Seascape (WHOI) data layer with a Manatee mortality GIS time series (FWRI). Portal available at http://axiomdatascience.com/maps/ioos/mbon/#module-search

CeNCOOS and GCOOS are coordinating efforts for designing a framework for assimilating and hosting MBON data and metadata in a fashion that is compatible with conventional standards (i.e. Darwin Core, Humboldt Core). The goal is to develop a system that is transferrable, as an operational infrastructure for the MBON.

Field data collections:

Significant progress has been made in the design and implementation of a field data collection strategy for developing pilot MBON efforts in the FKNMS and MBNMS. Three sites in the FKNMS have been selected as MBON 'anchor stations':

- Molasses Reef (25.380°N, -80.010°W),
- Looe Key (24.538°N, -81.413°W), and
- Western Sambo (24.478°N, -81.717°W; Fig. 2).

These will be sampled near-monthly to build a time series of biological and environmental observations for the MBON. The measurements include collection of eDNA samples. Two additional stations will be sampled opportunistically (Riley's Hump and Tortugas Bank; Fig. 2).

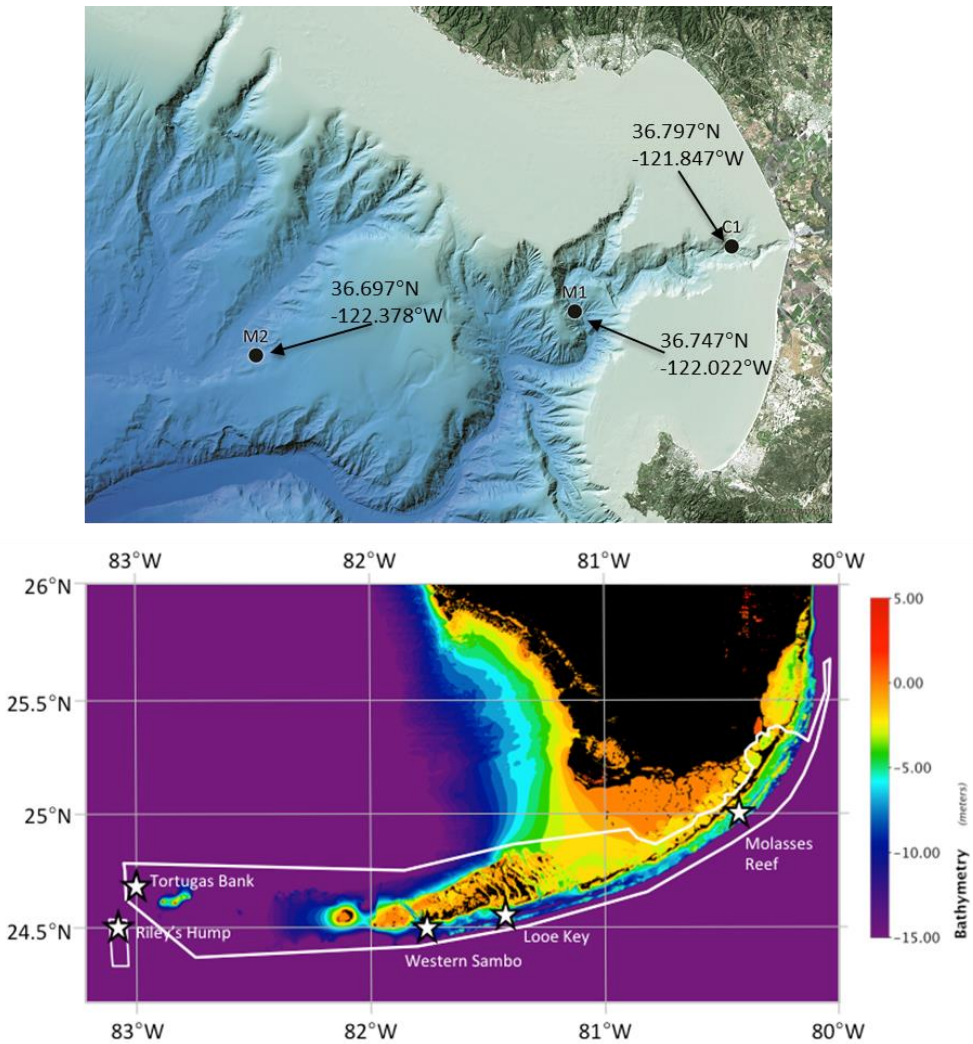

Fig. 2. Location of MBON stations in the MBNMS (top panel) and FKNMS (bottom panel). The three stations in MBNMS, and the three in the FKNMS (Molasses Reef, Looe Key, and Western Sambo), will be sampled near-monthly. Tortugas Bank and Riley's Hump stations will be sampled opportunistically.

Field data will be obtained at FKNMS by alternating sampling of these stations using small boats provided by FKNMS one month, and the following month from research cruises on the *R/V Walton Smith* (South Florida Program, AOML). To date, two research cruises aboard the *R/V Walton Smith* (U. Miami; April 12-17 and June 1-5) for the MBON have been completed. Field campaigns on small boats lead by the FKNMS science team (Scott Donahue) will be start in July 22-26. During these expeditions the team will collect data and samples and test the logistical requirements for maintaining a long-term sampling program in this region to serve the MBON.

All field sampling activities in MBNMS for the MBON will be coordinated in the framework of the Monterey Bay Time Series Program led by MBARI. Stations C1, M1, and M2 from the Monterey Bay Time Series Program have been assigned as the MBON 'anchor stations' for MBNMS. These sites are three of the six selected locations of the Monterey Bay Time Series, and have been occupied approximately monthly since 1992 (see Fig 2).

Genomics/eDNA:

The genomics/eDNA protocols for the FKNMS and MBNMS demonstration MBON are being developed. We have implemented an approach in which we parse out samples of DNA to four different institutions based on their interest and expertise. Specifically, nucleic acids are amplified with primers for vertebrates (12S) by Stanford, for plankton (18S/28S/C01) by the MBARI and for microbes (16S) by the USF team. The fourth team, at the Fish and Wildlife Research Institute (FWRI), will follow the protocols developed by MBARI to process samples for phytoplankton in the Florida Keys.

An initial focus has been to determine the best combination of filters and nucleic acid extractions to be used for MBON samples. This effort has been led by Stanford U. in collaboration with MBARI, USF, and FWRI. Samples were collected in the nearshore kelp bed ecosystem of the MBNMS and in the Florida Keys at the three coral reef sites. Water was filtered onto five different filter types (six filter types for the FKNMS) and the nucleic acids extracted using three different procedures. These are then analyzed by next generation sequencing at each institution as mentioned above. These analyses are ongoing.

To enable comparison of microbial communities across MBON locations, the USF group has coordinated with researchers for the Santa Barbara Channel and Chukchi Sea MBON projects. Together they have identified the conserved section of the 16S gene that would be most appropriate for analysis of the MBON samples and have selected primers to target that section. Amplification of the 16S gene is underway. The amplified nucleic acids for vertebrates, plankton and microbes are then analyzed using next generation sequencing and the results for the different filters extraction compared to determine the optimal combination.

Once methods are vetted, MBNMS samples will be analyzed by Stanford (vertebrates), MBARI (plankton), and USF (microbes). FKNMS samples will be analyzed by Stanford (vertebrates), USF (zooplankton and microbes), and the FWRI (phytoplankton).

A more detailed technical approach for the methods comparison experiments is given below. A summary of activities for the individual groups follows.

Experimental Approach:

The DNA samples are collected at FKNMS and MBNMS using the following methodology:

1. 75 liters of seawater are collected from a selected site (e.g. kelp bed, coral reef), homogenized and filtered using 1 L aliquots through five different filter types (PVDF, NC, PES, GF/F, PCTE; Fig. 3) in triplicate (four triplicate sets or n = 12 per filter type). Filtered samples are the stored at -80°C. An additional set of triplicate filters per filter type are included to serve as filtration blanks (filtering of 1L distilled water through the blank in parallel with sample filtering). Filter types are 0.2 um/47 mm except for GF/F which is 0.7 um/47 mm. A hand-deployed Niskin bottle is being used in MBNMS to collect water at depth (~10 m). In FKNMS, rosette-mounted Niskin bottles are used for sample collection at the surface and bottom (~ 20-30 m) from the *R/V Walton Smith*, while manual collection will be used for small-boat sampling at the same depths.
2. DNA will be extracted from the filters using three different methods, which include methods used in MBARI labs for bacterial and phytoplankton eDNA as well as those for

vertebrate eDNA. The three methods include: 1) MoBio PowerWater kit, 2) modified DNeasy Blood and Tissue kit (additional upstream lysis step) and 3) phenol-chloroform. Based on the frequent occurrence of inhibitory factors in DNA extracts from seawater, all samples will be further purified with Zymo One-step PCR inhibitor removal columns and eDNA will be eluted in the same volume and eluent. Total DNA will be quantified using the HS Qubit kit and eDNA extracts will be divided into three equal aliquots to be sent off to the different labs for further analysis: 12S (Stanford), 16S (USF), 18S/28S (MBARI; Fig. 3).

3. Next gen sequencing pipeline is applied and targets are quantified using QPCR to remaining DNA extracts. Protocols will vary by lab (Fig. 3).

Example Stanford next gen sequencing pipeline protocol for eDNA:
PCR: If necessary, DNA extracts are diluted at 1:10 or greater. DNA extracts are then amplified using tagged primers in quadruplicate and then the quadruplicate samples are pooled or not pooled depending on how we intend to use replicates. The Stanford team uses the HotStarTaq Plus Master Mix kit (Qiagen, cat # 203645) and load 0.5-10 ng of DNA per reaction. Cleanups are performed with the AMPure XP SPRI bead system. Samples to be sequenced should be pooled in equimolar concentration. The number of pools depends on the number of tagged primer sets available. As an example, for 84 samples, 28 tagged primer sets would require 3 separate pools.
For library preparations, Stanford uses a KAPA Biosystems library prep kit with barcodes from BIOO Scientific. 150-300 ng from each pool are used to build each library. The AMPure bead system is used for all clean up steps. If the concentration of the final library is <2.0 ng/µl a library amplification with 1-5 cycles is performed. Libraries are visualized and quantified using a Bioanalyzer.

Stanford uses a MiSeq instrument and has a custom data analysis pipeline.

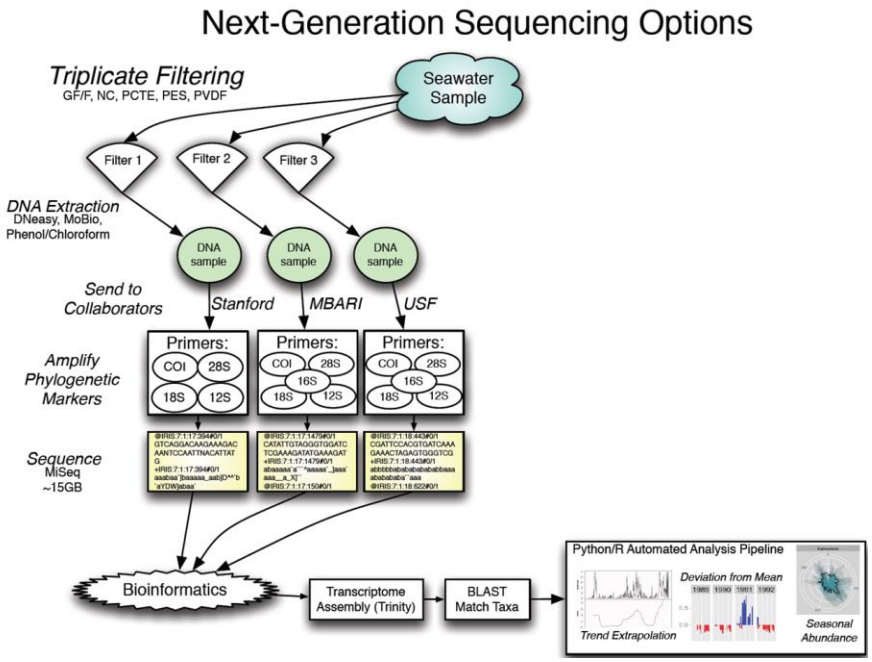

Fig. 3. Summary of MBON genomic/eDNA sampling and analyses.

Ongoing Team Activities:
Sample collection in the coral reef ecosystem of the FKNMS began in April 11-17 and continues approximately every eight weeks. A second set of samples was collected in June 1-5, with a third cruise scheduled for July 27-31. These collections are made at ten stations aboard the *R/V Walton Smith* (U. Miami), with sampling drawn from one or two depths (surface and bottom) at each station. To complement these bimonthly cruises, sampling at additional locations in the FKNMS will begin soon from a smaller vessel with the support of the FKNMS science team. The Stanford team has also begun DNA extractions for the MBNMS time-series at station M1. This time-series includes 7 cruises from June 2013 to October 2014. For each time point at M1, 1 L water samples were collected in triplicate at 11 depths spanning 0-200 m. The modified DNeasy extraction method will be used for these samples and DNA extracts then shared across the groups to profile how microbial, phytoplankton, zooplankton and vertebrate communities change with depth and season, and eventually over the long-term.

The MBARI team has begun extracting DNA from sample filters collected during the MBARI time series cruises. Monthly surface samples were collected from 3 stations in MBNMS from 2013 to the present. Samples are being extracted with the modified DNeasy extraction method developed by Stanford U. The DNA extracted from these samples will be sequenced for phytoplankton using the 18S primers. The relative abundance of sequences returned will be compared to corresponding phytoplankton counts by microscopy. These results will allow us to assess the precision of the relative abundance measurements of phytoplankton sequences compared to traditional microscopy methods.

FWRI has facilitated sample collection for phytoplankton analyses as part of the FKNMS cruises. Analysts are in the process of identifying taxa for comparisons with forthcoming eDNA data. The FWRI team has extracted eDNA from sample filters collected as part of a different ongoing time series project in the FKNMS. Samples were extracted (DNeasy) and 16S rRNA amplicon sequencing was conducted following approaches described above (i.e. MiSeq platform). These data are being used to evaluate and learn bioinformatics analysis approaches. The FWRI team also facilitated the collection of eDNA samples during a coral spawning event and will continue to provide opportunistic samples associated with wildlife collection and/or field response efforts.

Seascapes and high-res satellite remote sensing products:

Seascapes: Scott Doney and Maria Kavanaugh are working on an intercomparison of biogeographic region classification algorithms in space and time. To date, classifications that represent monthly biological-physical variability of chlorophyll-a (chl-a), phytoplankton fluorescence, SST, and salinity from 2003-2008 have been completed as a test bed to validate against in situ data in the FKNMS and MBNMS (Year 1; Fig. 4). These classifications are based on a modification of methods outlined in Kavanaugh et al. 2014.

Muller-Karger et al. (2015) used remote-sensing based observations of sea surface temperature (SST), wind speed, sea surface height anomaly (SSHA), chlorophyll-a concentration (Chl-a) and net primary productivity (NPP), along with model predictions of mixed layer depth (MLD), to determine seasonal changes and long-term trends in the central Gulf of Mexico between the early 1980's and 2012. Specifically, we examined variability in four quadrants of the Gulf of Mexico

(water depth > 1000 m). The analysis was needed to define baseline environmental conditions and trends in the past 20-30 years in the interior of the Gulf of Mexico. Understanding the background of seasonal and long-term variability in these ocean characteristics is important to interpret changes in ocean health due to episodic natural and anthropogenic events and long term climate changes or development activities. We are developing similar analyses for shallower areas of the Gulf.

Plans are now in place for spatial comparisons of krill data in the MBNMS, spatiotemporal comparisons of phytoplankton in the MBNMS, and an analysis of the spatiotemporal patterns of blue fin tuna recruits in the Gulf of Mexico, adjacent to the Florida Keys. The in situ comparisons will provide initial scholarly products and allow for better tuning of the seascape classification algorithm. (Year 2).

Kavanaugh will be working with Enrique Montes Herrera on development and application of satellite-based algorithms of phytoplankton functional groups in the two regions. Kavanaugh and Doney are already working on similar algorithms for phytoplankton groups along the Western Antarctic Peninsula for an independently funded project. As a first step, EMH is applying the algorithms to the FKNMS developed for coastal Antarctica (Kavanaugh et al., 2015), with the understanding that modification will be likely in optically shallow regions. Routines for gridding, processing and implementing several existing phytoplankton functional type algorithms from Level 2 ocean color data have already been created. We acknowledge that there are substantial risks involved with the development of new algorithms in complex coastal regions, both in the challenge of developing skillful algorithms as well as in relying on the continuity of satellite sensors and particular wavelengths. We are working with David Siegel (from the Channel Island MBON team) to implement existing algorithms for the Santa Barbara Channel based on the work of Kostadinov et al.(2007,2010) (Year 2-4).

The comparisons between seascape and in situ data will be developed in MATLAB. Metrics of success will be developed to see whether the DMAC can implement the algorithm to classify seascapes at higher frequencies, in real time, and with additional variables that characterize local conditions or not, and whether Sanctuary managers use this information after training. Kavanaugh will work with Science Coordinators and resource managers in the MBNMS and FKNMS to transfer codes and methods (Year 4 and 5).

Initial results are being shared among MBON affiliates through the Axiom workspace. We will also be working with the Biological and Chemical Oceanography Data Management Office (BCO-DMO) located at the Woods Hole Oceanographic Institution (WHOI) to archive all of our results. The BCO-DMO was established to serve the data management needs of biological and chemical oceanographers funded by the National Science Foundation.

The resulting seascape datasets will be a valuable tool for validating our conceptual models and numerical simulations being developed independently for Florida and California. Initially, all seascape intercomparisons and statistical summaries will be archived at WHOI. Our working group, the Computational Biogeochemistry group (CBGC) has several data servers available. A selection of validated seascape classification maps will be transferred to BCO-DMO for long-

term archival and by the wider community (Year 4 and 5). In the meantime, our results will be openly available via the Axiom/IOOS online workspace.

MODIS 250-m satellite products: Satellite data from the NASA Moderate Resolution Imaging Spectroradiometer (MODIS) sensor flown on the Terra satellite can be used to monitor suspended sediment plumes in coastal waters. MODIS has provided a time-series of satellite remote sensing observations at relatively high temporal resolution (near-weekly or better) and moderate spatial resolution (250 m pixels and coarser). Specifically, we generate time-series of water quality indices based on remote-sensing reflectance measurements at 645nm (Rrs645) using MODIS Band 1. The basic assumption is that sediments suspended near the water surface provide a signal in this red band, and that this signal can be measured from space distinctly from effects due to constituents in the atmosphere such as aerosols or reflection of light from the benthos. Measurements using MODIS band 1 are relatively insensitive to light reflected from the sea bottom in estuarine waters deeper than about 2.8 m due to the strong absorption of red light by water. An example image from 2010 is shown in Figure 5. In this image, Rrs645 shows evidence of sediments from Florida Bay being advected onto the Keys reef tract. This image was collected during a three-day period when winds blew from the northwest and wind speeds were ~2.5 m/s above average.

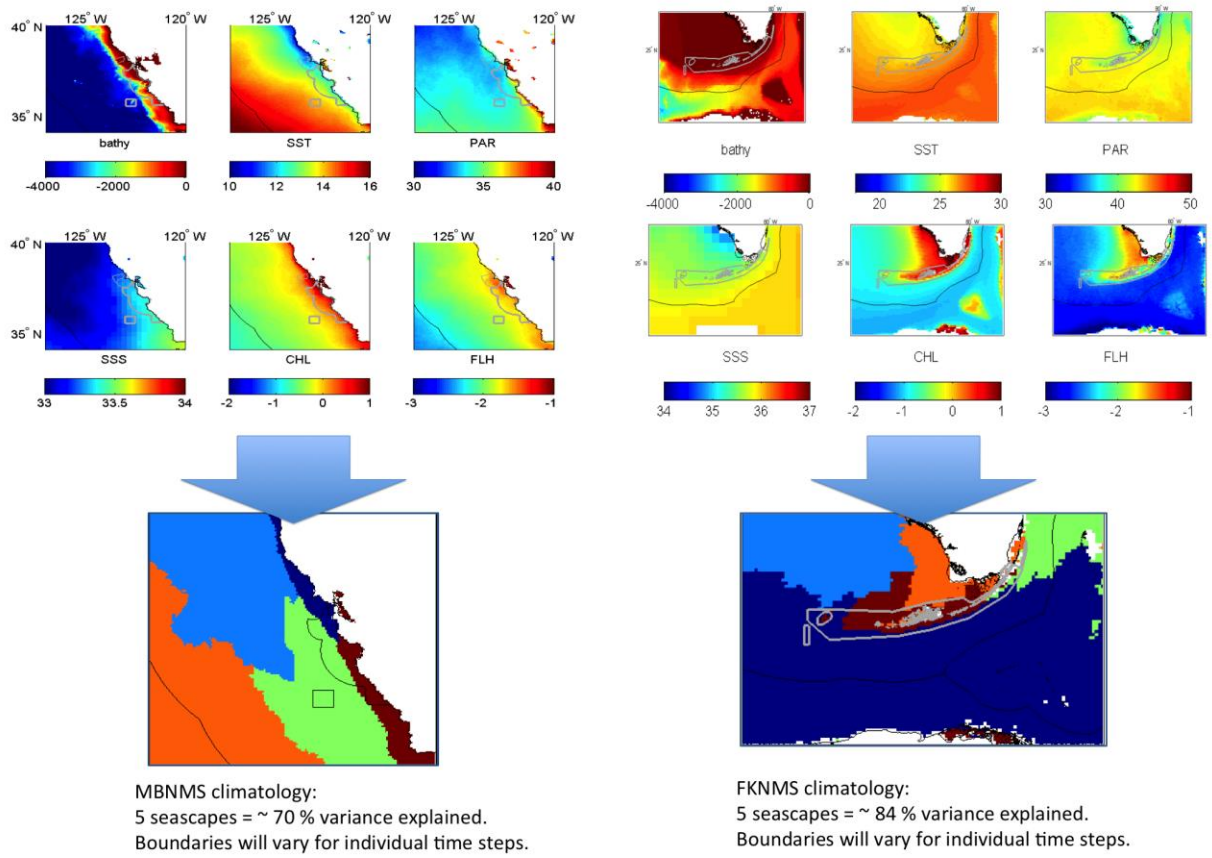

Figure 4. Seascape climatologies (2003-2008) for the FKNMS and MBNMS are shown in panels indicated by the blue arrows. The seascape distributions at each Sanctuary are derived from biophysical variability of parameters shown above the seascape panels (bathymetry, sea surface temperature (SST), photosynthetically active radiation (PAR), sea surface salinity (SSS), chlorophyll-a (CHL), and fluorescence line height (FLH).

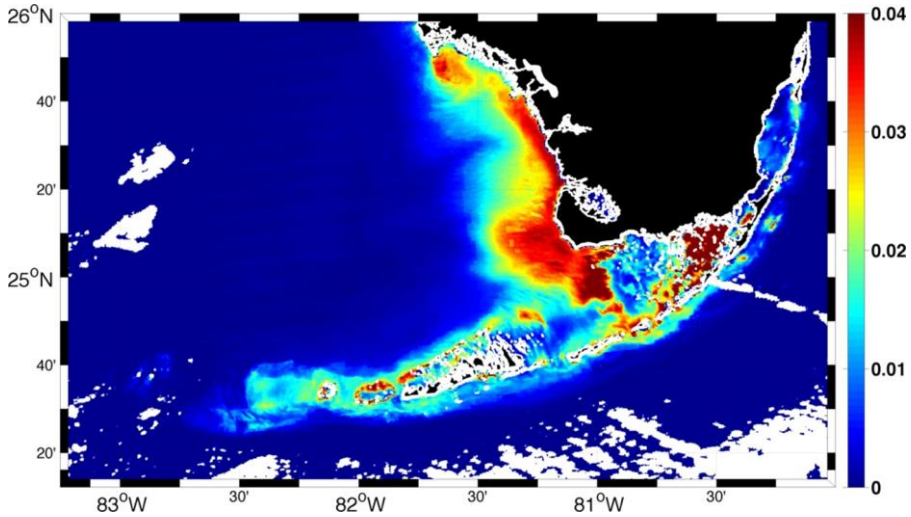

Fig. 5. MODIS Aqua image from March 13, 2010 showing Rrs (645nm), which is a proxy for turbidity. The spatial resolution of this band is 250 m. This image was collected during a period when winds were blowing from the northwest and wind speeds were ~2.5 m/s above average. Note the presence of suspended sediments along the reef tract in the middle Keys.

GEO BON MBON activities:

Muller-Karger was asked to lead a task team of the GEO Bon Working Group 5. The group has been meeting regularly over the phone. The team has developed a vision and specific objectives, as summarized below.

Vision: A global network of marine observation networks that monitors all key aspects of biodiversity-relevant change to support policy, decision making, and healthy and sustainable oceans.

Scope of MBONs:

- Voluntary global network of biodiversity observatories (i.e. entities that are long-standing or operational in character; i.e. not just one-time sampling)
- Broad, including anything that is marine, biological, and relevant to change
- Spatially local to regional, aggregated as a network with global scope
- Pan-taxonomic (microbes to whales)
- Includes pelagic, coastal, benthic, and demersal zones
- Includes ecosystems, species, and genes
- Includes ecosystem services

Goal: Facilitate collaboration, coordination, exchange of ideas, tools, and data, and the development of new MBONs in gap areas. (The perception is that GEOBON/WG5TT needs an actual, practical goal as well as funding to get anything accomplished.)

The present focus is to inventory existing long-standing/operational marine biodiversity observations. A separate objective is to understanding the diversity of sampling, data storage,

sharing practices, and develop a best practices document. This is envisioned as a possible contribution to BON in a Box.

Substantial amounts of time are going into the planning of a possible initiative at the GEO Ministerial meeting in Mexico City in November 2015, including contributions to MBON as well as AmeriGEOS side events.

PR and E&O activities:

The MBON E&O project coordinator, C.J. Reynolds is leading a joint planning effort with the National Marine Sanctuary program and Roffers Ocean Fishing Forecasting Services to create awareness of marine biodiversity research in context of the two sanctuaries. The plan is to create short articles and videos for the general public featuring activities conducted by MBON participants. The programs would highlight innovative research tools and emphasize the value of biodiversity. MBARI and GCOOS staff wrote press releases and short PR pieces for their electronic newsletters, which reach various scientific audiences. The E&O team will also work closely with DMAC to support outreach for the database and engage potential users. During the next year the team intends to highlight the value of remote sensing and other MBON products to stakeholders.

Digital/Video projects: The MBON E&O team is developing a plan for short videos (30-90 sec) describing fieldwork operations. Video footage, amateur and professional, is already being collected during expeditions to the Sanctuaries. The videos will be available online on various websites including ONMS' Earth is Blue site (http://sanctuaries.noaa.gov/earthisblue.html). One video showing fieldwork in the MBNMS featuring marine mammals assessments and eDNA sample collections has already been produced and is available online (https://www.youtube.com/watch?v=2B3Gz0rOg_A).

Website: The initial navigation plan was developed and will be discussed among Co-Investigators.

Publications/Presentations:

The MBON team has held several internal meetings to outline strategies to link research, education, and applications. These meetings include:

- West and east coast regional MBON kick-off meetings, January 15 and January 30.
- MBON All-hands kick-off meeting, March 2-4, 2015, Miami, FL

The MBON project and preliminary results have also been presented at:

- Office of National Marine Sanctuaries Research Coordinators' Retreat Meeting, September 22-26, 2014, Camp Letts – Edgewater, MD
- NASA Biodiversity and Ecological Forecasting Team Meeting, April 22-23, 2015, College Park, MD
- Future Earth meeting, May 4-6, Yale University, NJ
- Office of National Marine Sanctuaries Research Coordinators' Retreat Meeting, June 1-5, 2015; Galveston, TX
- Trait-based approaches to Ocean Life Workshop, October 5-8, 2015, Waterville Valley, NH (Two abstracts accepted).

The following meetings are now being planned:

- Cross-MBON meeting, October 13-15, 2015, Monterey, CA
- GEO Ministerial mtg and MBON side event / November 2015 / Mexico
- Ocean Sciences Meeting / 21-26 February 2016 in New Orleans, Louisiana.
    - GOOS BEP meeting
    - Accepted sessions:
        - Session Topic: Ocean Observing and Data Management
            - Session ID: 9374
            - Session Title: MBON Voyage: Integrating marine biodiversity into ocean observing systems
        - Session Topic: Marine Ecosystems
            - Session ID: 9538
            - Session Title: Observations of Climate Change and Marine Ecosystem Biodiversity

Peer-reviewed publications: The following MBON publication appeared in press

Muller-Karger, Frank E., Joseph P. Smith, Sandra Werner, Robert Chen, Mitchell Roffer, Yanyun Liu, Barbara Muhling, David Lindo-Atichati, John Lamkin, Sergio Cerdeira-Estrada, and David B. Enfield. 2015. Natural Variability of Surface Oceanographic Conditions in the Offshore Gulf of Mexico. Progress in Oceanography. 10.1016/j.pocean.2014.12.007.

Budget expenditures:

We have established subcontracts (seven) with partner institutions: Monterey Bay Aquarium research Institute (MBARI), Woods Hole Oceanographic Institution (WHOI), Center for Ocean Solutions (Stanford U.), Gulf of Mexico Coastal Ocean Observing System Regional Association (GCOOS-RA), ROFFS^{TM}, Fish and Wildlife Research Institute (FWRI), and Cooperative Institute for Marine Ecosystems and Climate (CIMEC - UCSC). A Postdoctoral Research Associate was also recruited and hired at the University of South Florida (A. Djurhuus). We are supporting one Master's graduate student (M. Hepner). PI Muller-Karger has 1 month summer salary charged to this grant.

Budget expenditures are on track. There are no changes to the budget for Year 2.

Additional NOAA contributions: NOAA has provided substantial contributions to the Sanctuary MBON project. Some of the contributions were not budgeted for in our original proposal.

Specifically, the IOOS office (G. Canonico) has supplemented the budget of the MBON project by $125,000. $25,000 was provided directly to Robert Bochenek/Axiom to expand the workspace domain to cover the Florida Keys, not just the west coast of the U.S. The IOOS office then provided a supplement of $100,000 to augment the cross-MBON interactions between the Sanctuary MBON, AK MBON, and Santa Barbara MBON, as well as the Tennenbaum MarineGEO projects.

The Office of National Marine Sanctuaries has provided substantial support in the form of personnel time (Steve Gittings and Mitchell Tartt). NOAA AOML invited MBON scientists to participate in the bimonthly oceanographic cruises to the Florida Keys National Marine

Sanctuary. Each cruise represents around $10,000 and the MBON project effectively uses the time offered on board to collect measurements that we had not possibly anticipated. The NOAA National Marine Fisheries Southwest Fisheries Science Center (SWFSC) provided in excess of $60,000 to cover time of personnel which had been requested in the original proposal but which could not be provided to the SWFSC. The director of the SWFSC offered to cover year 2 of their activities as well, at order of $62,000, if their budget allowed. There are likely many other NOAA resources put toward this program.

Also, the Florida Keys National Marine Sanctuary has provided logistical support in the form of boats, fuel, and personnel exceeding the approximately $50,000 requested per year in the proposal.

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