MBON Progress Report Year 3: 2016 – 2017

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

Cooperative Agreement Number: NASA NNX14AP62A

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SECTION 1: PROGRAM GOALS AND SUMMARY

Research Objectives:

Under this Cooperative Agreement, the project elements are administrated by the University of South Florida. The Cooperative Agreement includes 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) and the Global Ocean Observing System (GOOS - UNESCO).

Summary of Progress

Efforts during the third year of this Cooperative Agreement focused on continuing the development of a demonstration Marine Biodiversity Observation Network (MBON) to address these objectives. The demonstration is building capacity 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. The project is also deeply engaged in international activities including:

- GEO BON, by promoting a global MBON;
- AmeriGEOSS to organize a regional network in the Americas under the name Pole to Pole MBON in the Americas;
- GEO Blue Planet: MBON is considered the marine biodiversity arm of Blue Planet;
- Various United Nations efforts including:
  - In December, 2016, MBON signed a collaboration agreement with the Global Ocean Observing System (GOOS) and the Ocean Biogeographic Information System (OBIS). The goal of signing the agreement is to:

        - foster a partnership with the Intergovernmental Oceanographic Commission (IOC) to define Essential Biodiversity Variables (EOV) under the GOOS Essential Ocean Variables (EOV);
        - engage OBIS to expand the community that uses specific standards to use and share biodiversity data;
    - develop prototype products to assist states and researchers in addressing aspects of the UN Sustainable Development Goals, including SDG 14;
    - engaging in various opportunistic discussions (IPBES, CBD, AtlanTOS, etc.).

Our team effort continues to focus on the development of tools, concepts and best practices that contribute to assessments of ecosystem integrity, advancing protection and management of marine living resources, and promoting conservation across levels of society. During this funding cycle, the team continued to make substantial progress in the development of molecular eDNA techniques to evaluate habitat diversity and diversity of lower to higher trophic levels. This novel approach will also aid in the detection of significant change in biodiversity over time across Sanctuaries, and help identify invasive species. The team has advanced multivariate remote sensing techniques to evaluate dynamic 'seascapes' from regional to global scales to enhance the spatial footprint of in situ observations. The Sanctuaries MBON program has also made substantial progress in the development of a robust Data Management and Communication strategy for effectively aggregating and disseminating biodiversity data collected by a large number of independent programs to address scientific, management and policy challenges across the U.S. and abroad. This program is developed jointly with the IOOS Regional Associations (GCOOS and CenCOOS) and both the U.S. and the international OBIS program. There is an ongoing dialogue with other U.S. MBON programs to advance on a national biodiversity observation strategy.

The Sanctuaries MBON team continues to coordinate efforts and leverage resources with two other U.S. MBON demonstrations. The cross-MBON effort includes discussion on remote sensing (seascapes), eDNA, and data management. The Sanctuaries MBON is providing assistance and prototype products (seascapes, eDNA methods and sample processing) for the Alaska MBON and the Santa Barbara Channel MBON.

The Sanctuaries MBON is engaged internationally through the GEO BON and AmeriGEOSS, Blue Planet, Global Ocean Observing System, the Convention of Biological Diversity (CBD - UN), and the Ocean Biogeographic Information System (OBIS - UNESCO). During the third year of the program, the Sanctuaries MBON led the creation of the framework for the first regional MBON in the American continent. This included organizing workshops and collaborations with colleagues across the region aimed at the development of the *Pole-to-Pole MBON of the Americas* framework. This effort will require financial support to be sustained, as all nations except Canada in the American continent are very limited in how they can engage in a regional network that advocates for the use of high technologies to measure and compare biodiversity across large scales.

In this funding period, the Sanctuaries MBON program also initiated efforts for the development of a concept product to inform targets of the U.N. Sustainable Development Goal #14 as a contribution of the U.S. government to the Group on Earth Observations (GEO).

The program includes graduate education and public outreach efforts in coordination with the Sanctuary programs, IOOS, and various other regional NOAA outreach and extension programs.

Transition of the MBON to operations is currently under discussion in partnership with NASA, NOAA and the IOOS program. A positive outcome is a commitment by NOAA NESDIS to engage in discussions to host both U.S. regional seascape products at high spatial resolution (1 km) and global products at reduced resolution (possibly 4 or 9 km). This dialogue started in June 2017. MBON is also holding active discussions with the new NASA COVERAGE team at the Jet Propulsion Lab, after their COVERAGE concept was approved by CEOS in May 2017.

Expected products and Outcomes in Program Years 4-5:

1. Further development of conceptual models of ecosystem structure within each Sanctuary including key indicator groups.
2. A centralized data management plan that provides:
   a) integrated existing biodiversity information spatially and over time from each Sanctuary
   b) an implementation strategy for IOOS and Sanctuary programs.
3. A fully operational 'seascape' framework to integrate merged satellite and in situ observations in partnership with IOOS Data Management and Communication (DMAC) systems.
4. Coordination of sample collection, methods, and data integration into DMAC. This includes:
   a) the 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.
   b) the implementation of the framework into IOOS DMAC.
   c) a list of minimum observations required for an MBON for each Sanctuary.
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. 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.
   b) Design and implement an interactive online biodiversity geospatial information system in collaboration with OBIS; this will be the regional/local scale version of the product being designed to address aspects of UN SDG 14 (i.e. SDG 14.2 and SDG 14.a). The reasoning is that any resource manager that requires marine biodiversity information in any place around the world will have similar problems to address as those of the U.S. National Marine Sanctuaries. These problems are captured in the series of questions that guide development of the Sanctuary Condition Reports.
7. Evaluation of socio-economic value and policy options
   a) Annual information needs assessments in Monterey, FL Keys, Flower Garden Banks using smaller workshops and stakeholder surveys
8. Further develop the international dimensions of the MBON
   a) Consolidate plans for the GEO MBON concept, including refining the MBON implementation plan.

b) Advance planning on a Pole to Pole MBON pilot, specifically working with AmeriGEOSS in the Americas
c) Further develop the pilot interactive information tool to support targets of the U.N. Sustainable Development Goal 14.
d) Contribute to the convergence on GOOS Essential Ocean Biological and GEO BON Biodiversity Variables.
e) Participate in the development of marine elements to BON in a Box.
f) Participate in the BON Development Working Group and other GEO BON Working Groups.
g) Participate in GEO Blue Planet and other GEO initiatives and promote linkages between them.

SECTION 2: PROGRESS AND ACCOMPLISHMENTS

This report presents brief descriptions of accomplishments during Year 3 of the project according to the following table of content:

1. Gulf of Mexico Coastal Ocean Observing System (GCOOS) and Florida Fish and Wildlife Conservation Commission (FWC) Efforts

GCOOS Data Management and Communications (DMAC) Team Activities: Addition of Environmental Datasets to Florida Portal

The GCOOS DMAC teams have been assembling and reformatting environmental datasets for the Florida Keys MBON program. These datasets were produced by various organizations including NOAA's Southeast Fisheries Science Center, Florida's Fish and Wildlife Research Institute, the University of Miami, the Florida Department of Environmental Protection, and many others.

The GCOOS team has been transforming these datasets into NetCDF files along with comprehensive standardized metadata conforming to standards endorsed by the National Centers of Environmental Information (NCEI) NetCDF Feature Templates and OBIS-USA (U.S. Geological Survey). These transformations enable data to be served via the GCOOS ERDDAP server which provides data access for both people and automated computer programs. Datasets transformed by the GCOOS team include fish observations from the Florida Keys Reef Visual Census (RVC; 1994-2014), the Dry Tortugas Reef Visual Census (DT-RVC; 1999-2014), the Comparative Assessment of Gulf Estuarine ecoSystems (CAGES; 1998-2005) and the Florida Keys Coral Reef Monitoring Project (CREMP; 1996-2013), as well as water quality data (e.g. dissolved oxygen, nutrients, chlorophyll-a, salinity) for the Florida Keys from 1970 through 2013 extracted from the GCOOS Hypoxia-Nutrient Data Portal (Figure 1).

Over the next few months, environmental data from 31 field surveys (2006-2016) conducted by the University of Miami's R/V Walton Smith as part of the South Florida program (SFP), and historical and near real-time oceanographic and marine meteorological data from NOAA's National Data Buoy Center, water level data from NOAA's National Ocean Survey, and salinity and temperature data from the Everglades National Park will be added to the ERDDAP server (Figure 2).

Fish data from the Fisheries Independent Monitoring (FIM) program led by the Florida Fish and Wildlife Commission have been transformed by the SE Coastal Ocean Observing Regional Association (SECOORA) and will soon be available through the ERDDAP server. The Southeast Area Monitoring and Assessment Program (SEAMAP) fishery-independent datasets are being assessed for assimilation into the MBON program.

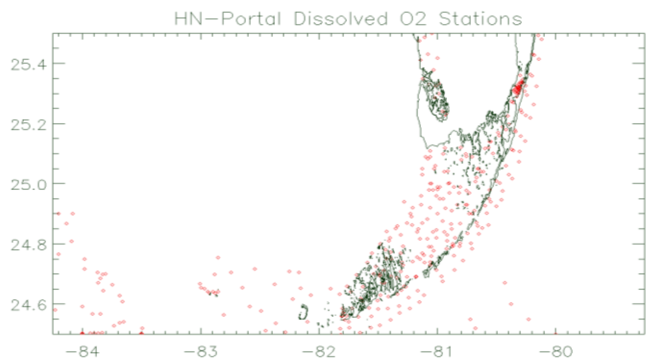


Figure 1. Locations of dissolved oxygen data from the GCOOS Hypoxia-Nutrient Data Portal.

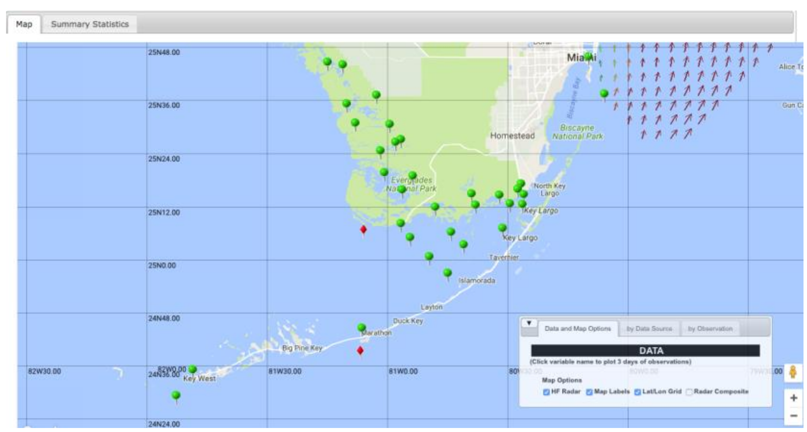


Figure 2. Station locations for NOAA's National Data Buoy Center and National Ocean Service, and Everglades National Park stations.

Florida FWRI DMAC Activities

This year, the Florida Fish and Wildlife Research Institute (FWRI) DMAC group worked to acquire all relevant data to aid in the study of biodiversity in the Florida Keys. We have collected fishing data from NOAA's Recreational Fisheries Statistics Survey (MRFSS) for use in the EcoSpace model as well as some FWC datasets that have never been disseminated for GIS use;

such as the Queen Conch Survey Sites. In all, the Fish and Wildlife Research Institute is now hosting 40 datasets and their associated tables. These are all available through FWC's Open Data Platform by easily found by using the search term "MBON". This platform allows users to easily search, visualize, filter, download and even create charts based on attributes (Bar, Donut, Line and Scatter Plot).

Currently available datasets for MBON are (added to or reformatted since last year's update are shown with an asterisk):

- REEF.org Fish Sightings and Abundance 1993-2000
- REEF.org Fish Sightings and Abundance 2001-2008
- REEF.org Fish Sightings and Abundance 2009-2015
- Multi Agency RVC Biomass 1994-2003*
- Multi Agency RVC Biomass 2004-2009*
- Multi Agency RVC Biomass 2010-2012*
- Multi Agency RVC Abundance 1994 -2003*
- Multi Agency RVC Abundance 2004-2009*
- Multi Agency RVC Abundance 2010-2012*
- Florida Shorebird Nesting Database 2011-2013*
- Beach Nesting Birds 2005-2010*
- Wildlife Observation Locations Florida*
- SECOORA In Situ Weather Observations
- Lobster Abundance Transects
- Timed Searched Lobster Abundance
- Postlarval Collectors
- Queen Conch Survey Sites
- Walton Smith Stations
- Middle Keys Nearshore Hardbottom Survey
- FIM Inshore SFL Subset
- FIM Offshore Tortugas
- FIM Middle Keys Seine Surveys Sample Locations
- FKNMS Observed Fish Aggregations
- Recent HAB Events (2014-2016)
- Terrapin Observation Locations in Florida
- Manatee Synoptic Survey
- Acropora Presence/Absence Locations in Caribbean
- Rare and Imperiled Fish
- Artificial Reefs Florida
- Patch Reefs SE FL
- Coral Reef Evaluation and Monitoring Projects in FL (CREMP, SECREMP, DRTO CREMP)
- National Benthic Inventory (NBI)
- Water Quality STORET EPA FL
- Proposed Sites
- Unified Florida Reef Tract Benthic Habitat

- Seagrass Florida
- Saltwater Marsh Florida
- Tidal Flats Florida
- Coral Hardbottom
- Florida Keys National Marine Sanctuary Boundary*

Tables:

- MiddleKeys_SeineSurveys_Samples
- NearshoreHardbottom_Algae
- NearshoreHardbottom_Fish
- NearshoreHardbottom_Lobster
- NearshoreHardbottom_MotileInverts
- NearshoreHardbottom_Surveys_master
- NearshoreHardbottom_SessileInverts

The team continues to enhance the [MBON-FKNMS]() JavaScript-based data viewer, our main GIS data visualization tool for the Florida Keys, and expand its use among various stakeholders. The map data viewer remains our preferred application for visualizing all MBON datasets and is now at version 2.2 of the ESRI Web App Builder. The data viewer includes custom filters for selecting species within the Reef Visual Census (RVC) data (Figure 3) so stakeholders can look at changes in abundance for a single species. It also includes a new selection tool (Figure 4) for acquiring statistics about any of the datasets and creating a new data layer based on the selection.

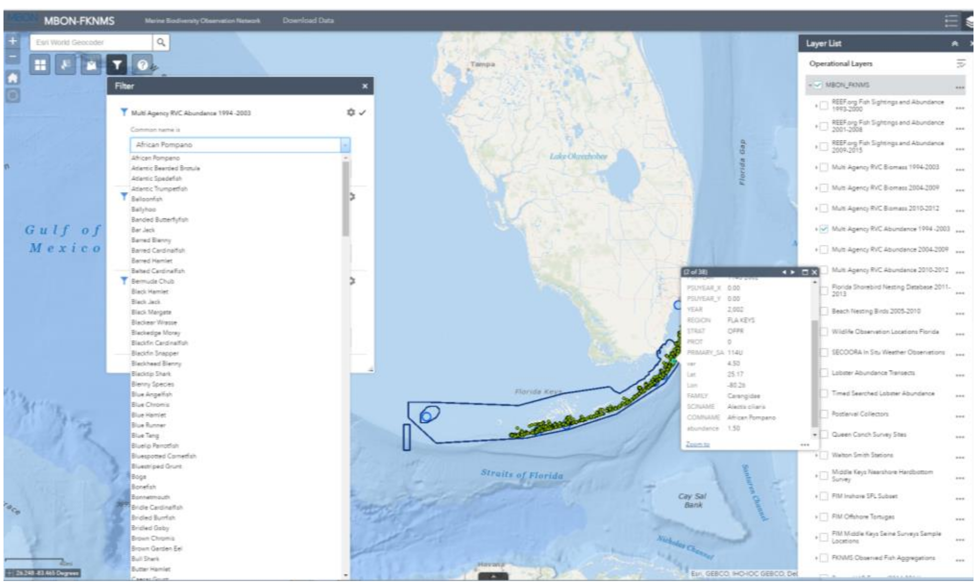


Figure 3: JavaScript Viewer RVC Species Data Filters

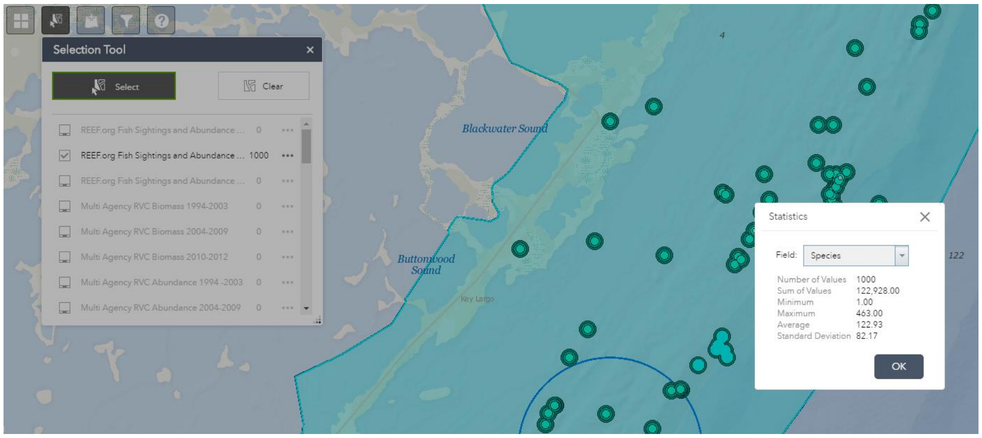


Figure 4: JavaScript Viewer Data Selection Tool with statistical functionality.

FWRI has ensured that all partners have access to relevant data layers and that they are in the correct format to function across all data portals. We have created a new webpage to allow easy access to information and updates on our continuing data management work and successful partnerships with other groups within the MBON team http://myfwc.com/research/gis/regional-projects/mbon/. We continue to develop new ways for researchers to access and work with our data as well as collecting new data that is being released from the eDNA team and updates to the current data sources.

FWRI Ecosystem Model Development

Coral reefs are one of the most speciose ecosystems in the world and are an extremely important economic resource for local communities, with high levels of productivity, yet generally occur in nutrient poor and plankton-poor oceanic waters (Lewis 1981). Coral reefs survive in seemingly unfavorable conditions due to a combination of complex species interactions, efficient nutrient recycling, and conservation (Opitz 1996). Understanding species interactions is increasingly important in complex reef environments, where a variety of mechanisms for species co-occurrence and distribution can occur.

As a complex interactive system, monitoring coral reefs from an ecosystem-based management perspective presents a significant challenge to natural resource managers. Monitoring single species within a coral reef generally does not account for larger scale regulatory dynamics such as anthropogenic pressure, competition, predation, habitat degradation, and natural perturbations (Heymans et al. 2014). On the other hand, deriving cogent information from all species simultaneously is often untenable. Increasingly, ecosystem models are used to fill the gap between overly simple and unwieldy ecosystem indicators to synthesize complex interactions into fundamental structural and functional components as a monitoring tool (Heymans et al. 2016).

The purpose of this study is to provide an ecosystem level monitoring tool for the Florida Reef Tract. The most common ecosystem modeling framework for the marine environment is the

Ecopath with Ecosim (EwE) suite (Colleter et al. 2014). The EwE model suite consists of a series of mass balance and delay difference equations to construct a food web that can be used to model ecosystem dynamics over time; the model assumes that the energy input and output of all living things must balance (Christensen and Walters 2004, Chagaris 2013). Initially established by Polovina (1984) to estimate biomass and energy flow between ecosystem components, EwE continues to develop and incorporate more complex dynamics.

Trophic groups in EwE are represented by aggregations of species or individual species of interest, which are initially governed by two master equations. First, the production of each functional group is defined as the sum of predation mortality, catch, net migration, biomass accumulation, and other mortality. Second, energy balance within each group is achieved by assuming that consumption is equal to the sum of production, respiration, and unassimilated energy. In addition to biomass, production, and consumption, the mass balance model requires the ecotrophic efficiency (EE) of each group, which represents the proportion of biomass from each group that is used in the system; EE is commonly estimated from the other three parameters (i.e., biomass, production, and consumption) (Christensen et al. 2008).

The data required to initialize EwE are often available from stock assessments, ecological studies, or the primary literature: biomass, mortality estimates, consumption estimates, diet compositions, and catch data. We derived biomass data for reef fish in the Florida Reef Tract from the South Florida Reef Fish Visual Census (RVC) multi-agency program. Biomass data were aggregated by trophic group, per year from 1994 to 2012, and averaged over a 2,151 km^2 area representing the sampling domain of the Florida Reef Tract from the Marquesas to Miami. Biomass for most non-fish trophic groups was estimated using EE values from existing regional food web models or estimates from existing literature (Thornhill et al. 2011, Hill et al. 2014).

The primary managers of the RVC database, the National Oceanographic and Atmospheric Administration (NOAA) National Marine Fisheries Services (NMFS), assisted with assigning 224 fish species to 19 fish trophic groups based on diet, mobility, behaviour, and taxonomy using established literature (Randall 1967), regional fish identification guides, expert opinion, and FishBase (Froese and Pauly 2006). Diet, mobility, behavior, and taxonomy were chosen to represent ecological roles representative of several species in aggregate to achieve broad inference in terms of ecological function (e.g., Purkis et al. 2008). Production, consumption, mortality rates, and diet composition were derived from other Gulf of Mexico and Caribbean food web models (Opitz 1996, Venier and Pauly 1997, Walters et al. 2008, Chagaris 2013).

Fishing fleets were separated by recreational and commercial catch, with sourcing from the NOAA marine recreational informational program and the Florida Fish and Wildlife Conservation Commission (FWC) dependent monitoring trip ticket program for Monroe County.
Results:

The Florida Reef Tract is an efficient system that is a net producer, at least on an annual basis, with a production to respiration ratio of 1.02 (Table 1). The total system throughput in 1994, or the amount of energy and carbon flowing through the food web, was estimated at 8,004 tons/km^2/year; of which consumption by organisms accounted for 51%. The system omnivory index (Christensen et al. 2008), a measure of the number of trophic levels consumers use in the system, is slightly lower than similar reefs at 0.19, but within a normal range (e.g., 0.23 for the virgin islands, Opitz 1996).

Table 1. Initial fit of the 1994 Ecopath model for the Florida Reef Tract. After mass-balance is achieved within an initial year, the model is fit to a time-series of biomass values to express changes in the system over time.

Parameter	Value	Units
Sum of all consumption	4121.372	t/km2/year
Sum of all exports	48.51654	t/km2/year
Sum of all respiratory flows	2737.12	t/km2/year
Sum of all flows into detritus	1097.189	t/km2/year
Total system throughput	8004.198	t/km2/year
Sum of all production	3701.197	t/km2/year
Mean trophic level of the catch	2.776938	
Gross efficiency (catch/net p.p.)	0.001879084	
Calculated total net primary production	2785.635	t/km2/year
Total primary production/total respiration	1.017725	
Net system production	48.5144	t/km2/year
Total primary production/total biomass	7.198873	
Total biomass/total throughput	0.04834392	/year
Total biomass (excluding detritus)	386.9543	t/km2
Total catch	5.234441	t/km2/year
Connectance Index	0.2146939	
System Omnivory Index	0.1920587	
Total market value	5.234441	
Total value	5.234441	
Total variable cost	4.187553	
Total cost	4.187553	
Profit	1.046888	
Shannon diversity index	2.261368	

FWRI Harmful Algal Bloom (HAB) Activities

Since the project's inception, the FWRI HAB group (Hubbard, Olesin) has prepared sampling kits for all Walton Smith cruises, and have >250 Lugol's preserved samples. In 2016-2017, 87 samples were examined and all HAB taxa were enumerated. Of those 87 samples, 13 were analyzed to determine the full community composition in a 3 mL subsample. To date, 97 different "taxa" have been identified, representing different levels of taxonomic resolution from species to family, depending on features that can be discriminated readily. A few non-specific classifications such as "detritus" were also recorded for those samples.

In addition, a taxa list for Monroe County was generated from the FWRI Harmful Algal Bloom Monitoring Database, using historical as well as contemporary observations, and annotated with differing levels of taxonomic classification to help with the analysis and interpretation of 18S sequences from phytoplankton in relevant eDNA datasets (see below). Current and future efforts are further focused on integrating phytoplankton taxonomic composition data (eDNA and/or traditional methods) with seascape analyses, pigment composition, and absorption spectra.

The FWRI HAB group also participated in numerous project meetings with the CA and FL eDNA groups. Lab space at FWRI was prepared specifically for the FL MBON eDNA extractions, and relevant extractions were staged and jointly executed by USF/FWRI (this is described further in the section *Standardized Methods for eDNA Collection, Extraction, and Analysis* of this progress report). FWRI also assisted with PCRs to confirm that the extractions were successful, and is working with the USF genomics team to prepare some of this work for publication. Discussion will encompass the potential of incorporating eDNA sampling into biodiversity surveys in the FKNMS.

As referenced in the section *Standardized Methods for eDNA Collection, Extraction, and Analysis*, an interactive web article describing the overall project approach in the FKNMS was prepared to assist with public and stakeholder outreach, and published online in the 2017 FWRI Programs document:
http://myfwc.maps.arcgis.com/apps/Cascade/index.html?appid=fae996592ba74da7bd6ddba3ee66b159

2. ***DMAC Efforts in the Monterey Bay National Marine Sanctuary***

All Sanctuaries have a need to better characterize the diversity of habitats and animals in the sanctuary and to determine if any changes in the condition of those resources are due to natural or anthropogenic pressures. In the 2016-2017 year, we made great progress in developing a variety of products that are going to improve our ability to characterize and understand the status and trends of biodiversity in sanctuaries, as well as climate and ocean drivers and human pressures on those resources.

Development of Conceptual Ecosystem Models and Interactive Data Visualization Tools for Condition Report Assessments and the California Current Integrated Ecosystem Assessment

MBON team members from MBNMS, along with CINMS and the Office of National Marine Sanctuaries (ONMS), have developed the concept of using interactive visualizations to access key data products relevant to the questions in Sanctuary Condition Reports. The purpose of these new tools is to improve real-time availability of status and trend information and to increase accessibility to key data sets. The conceptual models, developed as 'infographics', are now being developed for the Florida Keys National Marine Sanctuary as a test pilot to see whether they also meet the need of local Sanctuary and other resource managers. These interactive visualizations will serve as a portal to data products that will support dynamically-updated sanctuary Condition Reports which is a core goal of the Sanctuaries MBON project.

The approach is based on visual conceptual models created by MBNMS in collaboration with the **California Current Integrated Ecosystem Assessment** (Greg Williams and Su Kim). We recently integrated reviewer comments into updated versions of the conceptual models for the eight major habitats in MBNMS as well as an overview (Figures 5 and 6).

An Infographics Working Group was created at the Sanctuary MBON meeting in March 15-17, 2017, focused on delivering products to support the United Nation’s Sustainable Development Goal 14 (SDG14; Life Below Water). This group is leading the development of habitat-based conceptual models for key habitats in Florida Keys NMS. In addition, Jennifer Brown (MBNMS) is collaborating with the research team at CINMS and the CCIEA team to customize the MBNMS infographics for CINMS.

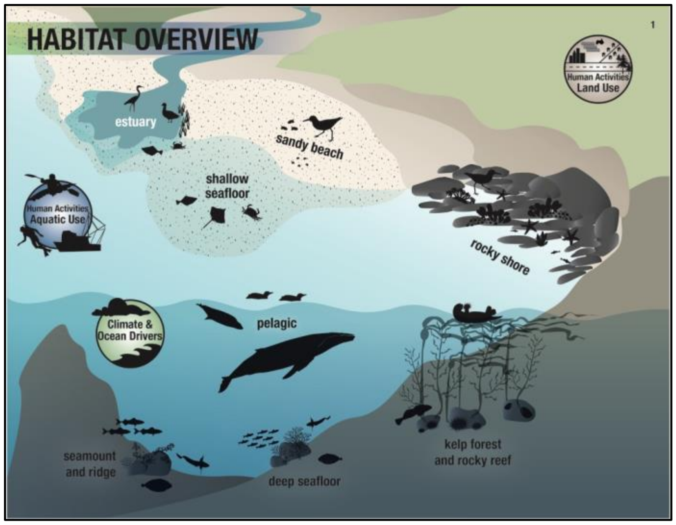


Figure 5. Overview conceptual model illustrating the eight major habitats within Monterey Bay National Marine Sanctuary, and icons representing land-based pressures, human activities, and climate and ocean drivers.

The approach to turn static conceptual models into interactive infographics is being piloted in 2-3 sanctuary-habitat combinations:

1) Monterey Bay NMS pelagic habitat (based on conceptual model above)
2) Florida Keys NMS coral reef habitat
3) Channel Islands NMS kelp forest habitat

These pilot interactive examples are being developed by Ben Best (EcoQuants) and available at https://marinebon.github.io/cr-metrics/pelagic.html. When a user clicks on a silhouette, the time series and status charts pop up (Figure 7). The goal is to enable a broad range of people – from decision makers to educators and engaged citizens – to identify species (or other targets) of interest and, with one or two clicks, see a snap shot of their current status and change over time. The visualization infographics are currently being revised and when ready, the online products will be accessible through various NOAA program websites.

This infographic product is one of a suite of online MBON applications whose technical development is being led by Ben Best in collaboration MBON and Sanctuary scientists for interactive exploration of various data types: taxonomic, seascape, eDNA, and connectivity. The infographic approach for exploring taxonomy will be applied to MBON sites (Florida Keys, Monterey Bay and Channel Islands) as well as globally using OBIS data and summarizing by country and protected areas. The Seascape explorer will display seascape categorical data in a map as well as time series of relative concentrations for regions of interest (ROI). Continuous data, such as SST and chlorophyll will be displayed with measures of spatial heterogeneity over time for ROI. The eDNA explorer will be applied to the FKNMS and MBNMS datasets (Fig. 8).

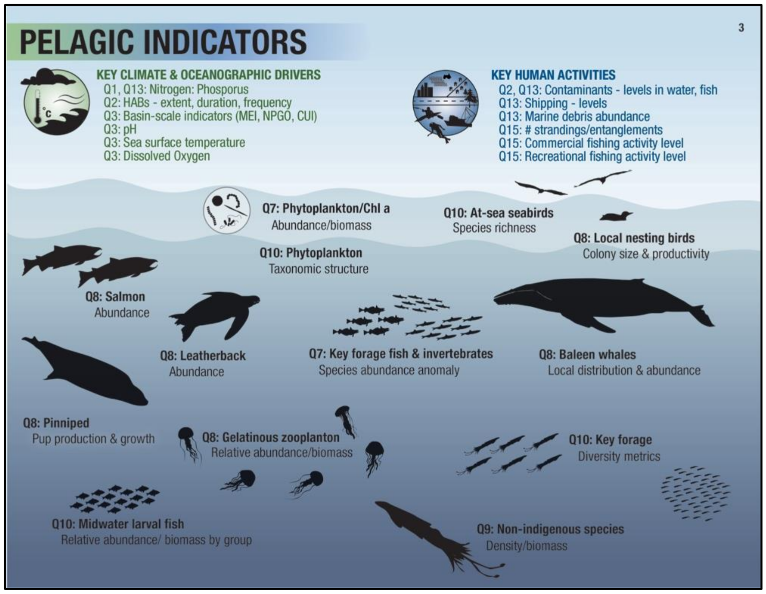


Figure 6. Conceptual model depicting the portfolio of indicators of ecosystem components (black font), climate and ocean drivers (green font), and human pressures (blue font) for the pelagic habitat in Monterey Bay National Marine Sanctuary. Q numbers align with the standardized questions in Office of National Marine Sanctuaries condition reports.

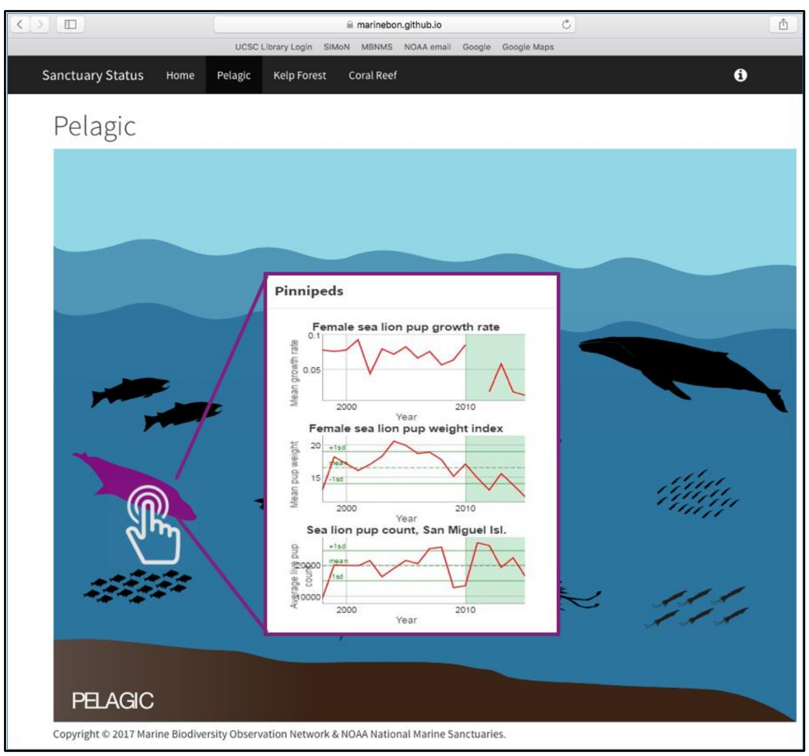


Figure 7. A print-screen from the demonstration website under-development by the Sanctuaries MBON project to inform National Marine Sanctuaries status assessments using an interactive infographic approach for displaying habitat-based elements that link to time series data.

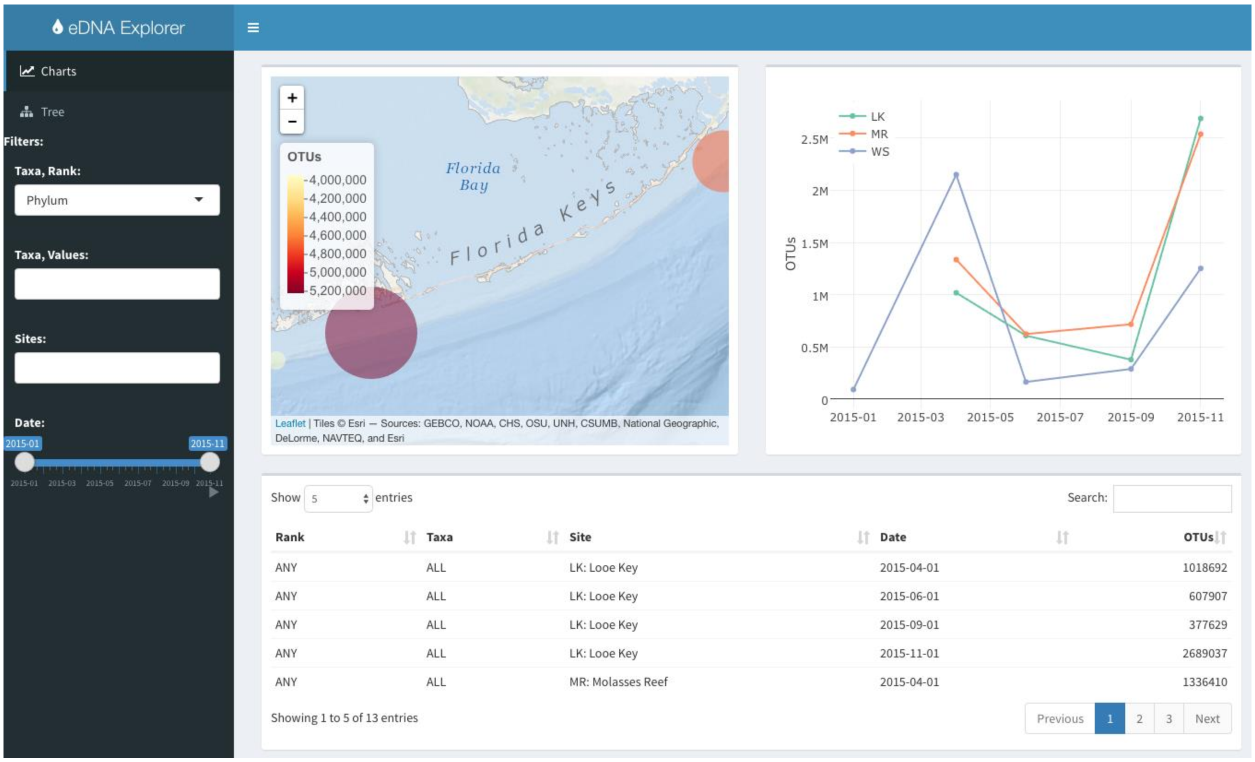


Figure 8. The eDNA explorer app to visualizes operational taxonomic units with filtering available by space, time and taxa and is available at https://marinebon.github.io/edna-vis.

Ben Best is in the process of creating a Connectivity Explorer app this summer of 2017, with UCSB Bren School student Stephanie Gad. She completed the technical analysis for Gray's Reef master's group project at UCSB's Bren School under Ben Best's advisement. The project yielded a digital products (https://graysreef.github.io) including interactive maps and animations that describe source locations of fish larvae based on sea surface current patterns, target species spawning locations and times. These source locations act as potential areas for additional protection to ensure healthy recruitment of fish populations to the sanctuary. Similar products will be generated for other sanctuaries, prioritizing Florida Keys, Monterey Bay, Flower Garden Banks and Channel Islands.

We will work with sanctuary managers and scientists to ensure the best available data are used and sanctuary-specific concerns are addressed. We will consider using habitats as proxies for known spawning locations, and representative spawning dates to capture variation between seasons and years with varying oceanographic regimes (e.g. El Niño vs. La Niña vs. normal). We will use Marine Geospatial Ecology Tools and contribute open-source functions (e.g. to create maps of cumulative percent larvae, and plots of area vs. percent larvae). A final report and digital datasets will be posted to MBON outlets, as well as manuscript drafted for submission to a peer reviewed journal.

We have identified key data sets that link to condition report questions that are the target for both icon development and for incorporation into data services and design of sanctuary-specific products (maps and/or time series). Key physical and biological time series for the MBNMS pelagic infographic include:

- basin-scale oceanographic indices (e.g., PDO, NPGO, ONI) (lead: Schroeder et al.);
- seascapes (lead: Kavanaugh et al.);
- SST and other satellite-based physical parameters (lead: CeNCOOS);
- temperature, DO and other platform-based physical parameters (lead: MBARI);
- phytoplankton (MBARI);
- HABs (CeNCOOS);
- krill (RREAS, MBARI, CIMT, ACCESS?),
- forage species by taxa and by biodiversity indices (NMFS-RREAS),
- seabirds nesting success (ACCESS/Point Blue and Oikonos),
- seabird and mammal sightings (NMFS RREAS)
- midwater assemblage (MBARI)

We have also identified some data sets linked to human activities that are good targets for incorporation into data services:

- vessel AIS (U. S. Coast Guard)
- whale entanglement reports (NOAA Fisheries Protected Resources Division)
- seabird and marine mammals strandings (Beach COMBERS, Marine Mammals Center/SCOOS)

After incorporation into data services, we will with data providers to design time series products that can be dynamically generated as new data become available.

Tracking Document for Monterey Bay MBON Education and Outreach

Jennifer Brown continues to track education and outreach efforts primarily for the Monterey Bay portion of the Sanctuaries MBON demonstration project. The tracking document contains information on presentations, publications, and press coverage. The Google document is shared with the members of the MBON team and all team members are encouraged to add entries. The Google documents is available here https://docs.google.com/a/noaa.gov/document/d/1v6-kPElFHRgVIBLqYNseBZqLfcO25zh77Bk5OmDOStI/edit?usp=sharing

Outreach to and Collaboration with the ***California Current Integrated Ecosystem Assessment*** *(CCIEA)*

As an active collaborator with members of CCIEA science team, MBNMS helps provide periodic outreach to the larger CCIEA group on MBON processes and product development. For example, Jennifer Brown periodically meets with the CCIEA web data visualization working group to share information and ideas for better web-based data visualizations. During the February 2017 meeting of this group, Brown presented the concept of the web-based data portal for dynamic sanctuary condition assessments, with early-stage demonstration of the MBON interactive infographic. Additionally, Jennifer periodically attends the monthly conference call of the CCIEA program and highlights opportunities for cross-program collaboration between sanctuaries, the CCIEA, and MBON. During the May 2017 monthly meeting, Gabrielle Canonico, Isaac Schroeder, and Jennifer Brown gave short informational presentations about the MBON demonstration program. Jennifer provided an overview of how MBON and CCIEA are both helping to provide status and trend data for sanctuary condition reports and progress on the interactive infographic concept to help display that data.

3. ***MBON Field Campaigns***

Expeditions to the Florida Keys National Marine Sanctuary (FKNMS) and surrounding waters

Multiple sites within the FKNMS and surrounding waters were sampled during Year 3 as in previous years of the project (Figure 9). Specifically, MBON ‘anchor stations’ in the FKNMS (Molasses Reef, Looe Key and Western Sambo) were occupied monthly to build a time series of microbial, bio-optical, genomics, and environmental observations, using small boats provided by FKNMS and aboard the *R/V Walton Smith* (South Florida Program, AOML).

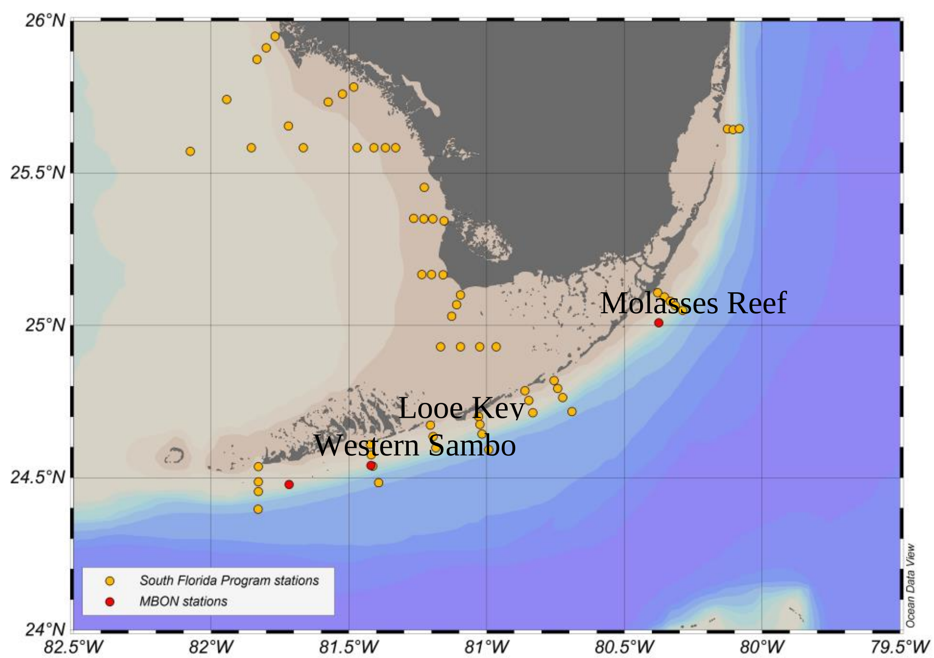


Figure 9. Location of the MBON and South Florida Program stations

Fort this annual reporting period, as of May 2017, six research cruises aboard the *R/V Walton Smith* and three field campaigns on small boats lead by the FKNMS science team for the MBON have been completed as follows:

May 9-13, 2016; *R/V Walton Smith*
July 25-29, 2016; *R/V Walton Smith*
September 19-23, 2016; *R/V Walton Smith*
November 14-18, 2016; *R/V Walton Smith*
January 30 – February 3, 2017; *R/V Walton Smith*
March 27-31, 2017; *R/V Walton Smith*

Additional sampling was conducted in the intervening months from small boats operated by the FKNMS. An additional set of samples was collected in a collaboration with the NOAA Genomics project (Jim Hendee, Kelly Goodwin) by Chris Sinigalliano (AOML) for eDNA analyses at Cheeca Rocks, to complement dissolved inorganic carbon and CO_2 sampling ongoing at this site by AOML as part of the NOAA Ocean Acidification monitoring program.

Seascape Validation Experiment in South Florida Waters

Since early 2016, sampling efforts have been focused on collecting observations that will assist in identifying relationships between in situ biodiversity of lower trophic levels and distributions of various seascape types. We also seek to validate the seascape classification model using in situ observations of the various constituents that affect inherent and apparent optical properties of the FKNMS, FL Bay, and the West FL Shelf. Sampling different seascapes will allow us to examine how biodiversity of different trophic levels is influenced by changes in ocean chemical and physical properties derived from ocean color data.

The seascape classification for south Florida waters is currently being improved with the use of satellite Chl-a input fields from an algorithm developed for shallow and optically complex coastal environments (Wattelez et al., 2016). The shallow water Chl-a algorithm (SW Chl-a) performs significantly better than the standard OCx Chl-a in this region. The SW Chl-a significantly improves the classification, especially over the shallow shelf area (Figure 10).

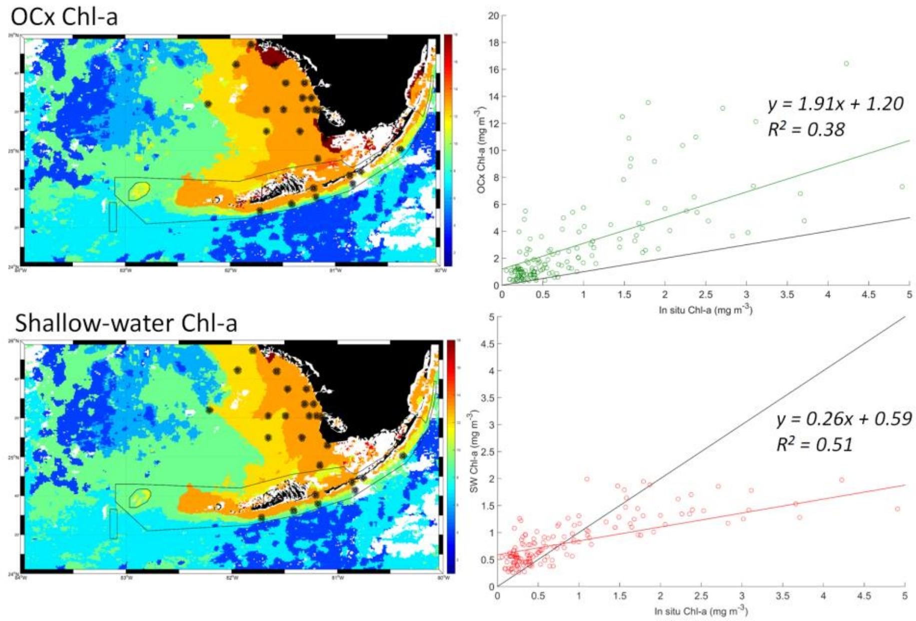


Figure 10. Maps show 8-day (March 11-18, 2016) composites of seascape distributions (MODISA 1-km) with classifications made using the standard Ocean Color (OCx) and shallow-water (SW; Wattelez et al., 2016) Chl-a algorithms.

Preliminary results show that phytoplankton pigment distributions and specific absorption spectra (a_{phy}*) spectra are strongly related to environmental conditions (salinity and NH_4^+ levels) and seascape type (coastal vs. oceanic; Figure 11). This indicates that phytoplankton assemblages in oceanic seascapes (≤ 11: high-salinity, low nutrient) are different from those in coastal seascapes (>12), which highlight areas more influenced by run-off. Seascapes > 14 highlight phytoplankton bloom (i.e. HAB's) conditions reported by the Florida Fish and Wildlife Conservation Commission (FWC). These results aid in the ecological interpretation of varying seascape distributions in south Florida waters and yield promising results on the utility of satellite seascapes for tracking areas dominated by HAB's in coastal ecosystems.

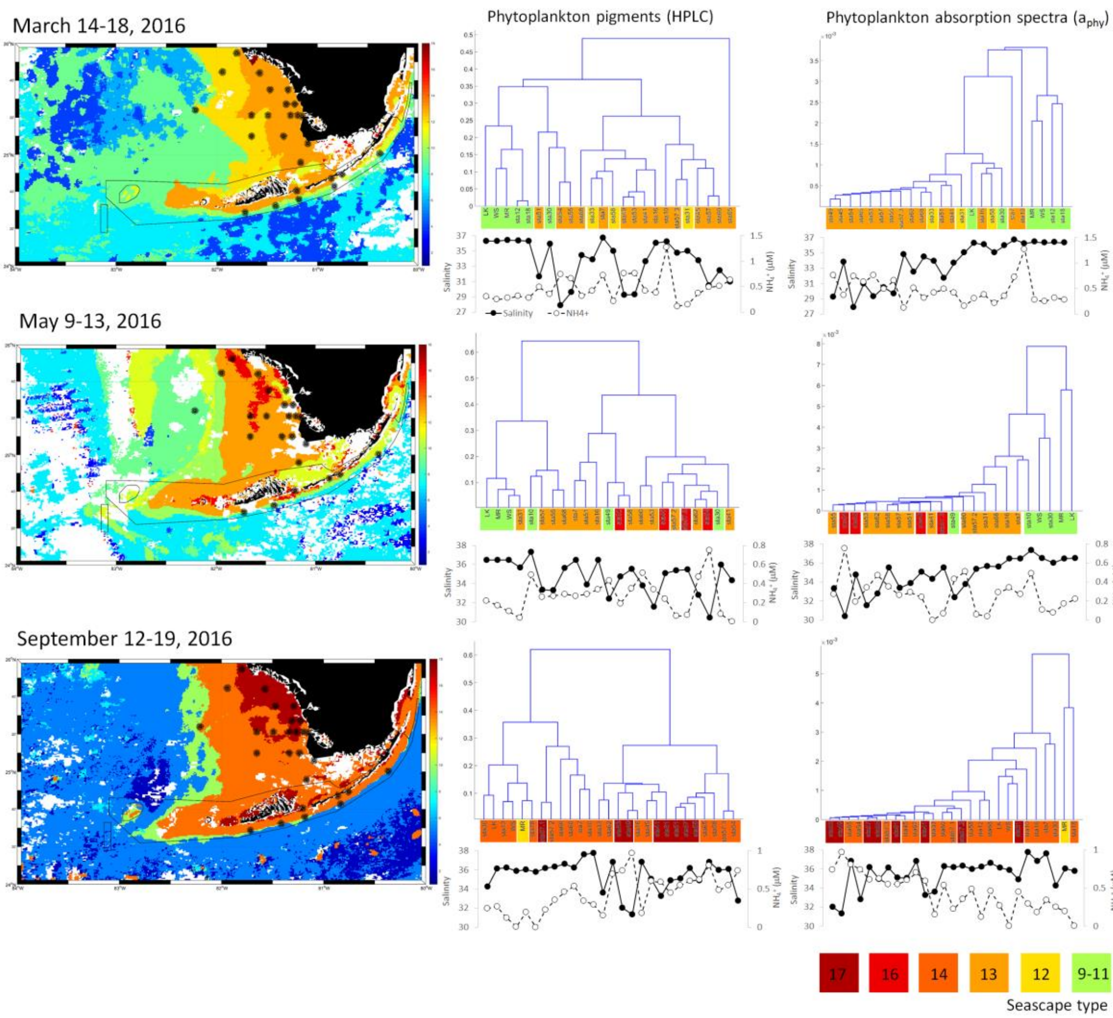


Figure 11. Seascape classification maps during three MBON - South Florida Program cruises are shown on the left. Sampled stations are highlighted with stars on each map. Dendrograms constructed with a Hierarchical Agglomerative Clustering (HAC) algorithm show classification of stations sampled for corresponding periods. Phytoplankton pigments (HPLC) and a_{phy}* spectra collected at each site were used as vector fields for HAC classifications. Plots below each dendrogram show salinity and ammonium levels for each station. Colors on labels in each dendrogram indicate mean seascape type for each station for a particular sampling period.

Environmental DNA and Microbial Assessments in the FKNMS

Various Sanctuary MBON groups continue to participate in bimonthly sampling cruises on board the R/V Walton Smith (Figure 12). In addition to collecting environmental DNA (eDNA) samples at the core sites (Molasses Reef, Looe Key, Western Sambo), we have been working with Maria Kavanaugh to identify and collect samples along seascapes. We have collected seascape-informed samples for multiple seasons, some of which will be selected for the microbes-to-whales analysis (see below). From several of these samples, including the outflow from Shark River, we will be performing metagenomic analyses of the microbial and viral communities in addition to the group-specific amplicon analyses. In total, we have over 1000 samples ready for analyses, and have shifted our focus towards strategic processing of these samples. We have also collected samples of opportunity from the Flower Gardens Banks in August 2016, after the June-July 2016 die-off of invertebrates.

Figure 12. (Left) USF MBON researchers Anni Djurhuus and Natalie Sawaya collect eDNA samples on board the R/V Walton Smith in September 2016. (Right) Kate Hubbard (FWRI), Anni Djurhuus (USF) and Natalie Sawaya (USF) extract DNA from these samples (with great enthusiasm!)

Standardized Methods for eDNA Collection, Extraction, and Analysis

The Sanctuaries MBON eDNA team, including Ali Boehm's group at Stanford, Francisco Chavez's group at MBARI, Mya Breitbart's lab at USF, and Kate Hubbard's HAB group at FWRI, completed method tests to determine the effects of different filter types and DNA extraction methods on diversity of bacteria (16S rDNA), phytoplankton (18S rDNA), invertebrates (COI), and vertebrates (12S rDNA). We have now identified several protocols that are acceptable for collecting and processing eDNA samples suitable for cross-trophic level analysis and developed a standard operating protocol. Other notable methodological progress includes the establishment and effective utilization of a common bioinformatics pipeline for analyzing all genetic markers. A manuscript describing this work is currently submitted at the journal Ecology and Evolution: Djurhuus, A, J Port, C Closek, K Yamahara, O Romero, K Walz, D Goldsmith, R Michisaki, M Breitbart, A Boehm, F Chavez (in review). Evaluation of filtration and DNA extraction methods for eDNA biodiversity assessments across multiple trophic levels.

Microbes to Whales: Cross-Trophic Level Analysis

We have made tremendous progress towards one of the main goals of this MBON project, namely a cross-trophic level analysis including bacteria, phytoplankton, zooplankton, invertebrates, and vertebrates, from both the Florida Keys and Monterey Bay sanctuaries. For a subset of samples capturing spatial and temporal variability from each sanctuary, eDNA has been collected and extracted and the following genes are currently being amplified and sequenced: the 16S rRNA gene for microbes (bacteria & archaea), the 18S rRNA gene for phytoplankton, the cytochrome oxidase I (COI) gene for invertebrates, and the 12S rRNA gene from vertebrates. All data will be processed through a common pipeline and statistically analyzed together.

Environmental DNA (eDNA) Sequencing Provides a Window Into Biological Diversity in the Florida Keys National Marine Sanctuary

We are currently preparing a manuscript describing 18S rDNA and COI sequences generated from eDNA collected from the three main stations in the FKNMS at four time points (April, June, September, November 2015). This manuscript will compare three biological replicates from each of the different genetic markers, the three different reefs, and the four seasons. These data reveal the wealth of taxa that can be identified using eDNA analysis from the smallest phytoplankton in the oceans to large organisms such as sea turtles and sharks. Since zooplankton (primarily arthropods) dominate both gene data sets, we have also performed detailed zooplankton analyses (see below). Discussion will encompass the potential of this molecular species inventory approach for describing biodiversity and how these data compare to our prior knowledge of diversity in the FKNMS. From these data, we expect to make recommendations for the amount and frequency of sampling and sequencing that needs to be done moving forward to obtain diversity estimates in a resource-effective matter. This manuscript is being led by PhD student Natalie Sawaya at the University of South Florida. An interactive web article describing the overall project approach in the FKNMS was prepared to assist with public and stakeholder outreach, and published online in the 2017 FWRI Programs document.

Comparing Diversity Estimates of Florida Keys Zooplankton Using Traditional Microscopic Versus Environmental DNA Methods

This completed study, led by USF postdoc Anni Djurhuus, compared three different methods used to assess zooplankton diversity: (1) microscopy versus amplicon (18S rDNA, COI) sequencing of (2) zooplankton tissue, and (3) environmental DNA (eDNA). The first two methods are collected directly from zooplankton net tows while eDNA is collected from the surrounding water, thus allowing researchers to compare eDNA to the physical presence of zooplankton. We also performed surveys with and without a pre-filter on the eDNA samples to determine the influence of copepod nauplii being caught on the filter. The preliminary results from this study, which demonstrated that most families seen through microscopy were also detected through eDNA sequencing, were presented by Anni Djurhuus as a talk at the 2016 Ocean Sciences Meeting. Further samples have been collected and processed for an in-depth, statistically robust taxonomic validation of zooplankton biodiversity as determined with eDNA vs. microscopy methods. We have now completed the full study and bioinformatic analysis of the sequence data. We expect this to yield a manuscript for submission within the next two months, which will be one of the first studies to groundtruth eDNA for documenting pelagic zooplankton diversity in the oceans.

Seascape-Informed Analyses of Microbial and Viral Diversity in Southwest Florida and the Florida Keys National Marine Sanctuary

Satellite seascape maps created by Maria Kavanaugh were used to guide the sampling during the March and September 2016 cruises. These maps (8-day composites) were developed for the week before the cruise and during the cruise to study the spatiotemporal evolution of seascapes across the region. Four seascape types were sampled across 27 stations on each cruise for hydrography, eDNA, phytoplankton taxonomy, and bio-optical properties. Seascape distributions were compared to concurrent underway observations and discrete observations of chlorophyll-a concentrations, specific absorption of phytoplankton, detritus, and colored dissolved organic matter (CDOM), and harmful algae counts from the Florida Fish and Wildlife Conservation Commission HAB program. Metagenomic sequencing of microbial and viral samples, 16S rDNA and 18S rDNA amplicon sequencing, and targeted flow cytometric and microscopy analyses for prokaryotes/eukaryotes, is currently underway and Anni Djurhuus presented a poster on this research at the 2017 Ocean Sciences Meeting.

Analysis of historical and current satellite and oceanographic cruise data of the northern Gulf of Mexico to evaluate the causes of a mortality event that affected the Flower Gardens Bank National Marine Sanctuary in June and July, 2016

The Sanctuaries MBON team engaged with the Flower Gardens Bank National Marine Sanctuary, NOAA Atlantic Oceanographic and Meteorological Laboratory, and Texas A&M University immediately upon Sanctuary personnel detecting a die-off in the organisms of the coral community in in June and July, 2016. The background research on possible causes for this event continue at this time. The Sanctuary MBON project analyzed an extensive series of satellite images that suggest the event was caused by a combination of anomalous rainfall over Texas in the preceding two months and subsequent runoff, and a coastal upwelling event that advected this coastal water over the Flower Garden Banks. The Sanctuaries MBON hypothesis is that this nutrient- and organic-matter rich water led to formation of hypoxic plumes or layers at the bottom of the surface low-salinity plume. The hypothesis is that mortality occurred when one of these hypoxic layers intersected the crown of the coral banks. The hypothesis is being tested using historical oceanographic cruise observations of salinity and oxygen, and time series of historical ocean color data collected from satellites.

Expedition to the Flower Gardens Bank National Marine Sanctuary during a coral spawning event

The USF team joined an expedition coordinated by the Flower Garden Banks National Marine Sanctuary (FGBNMS) to the East and West Flower Garden Banks during August 24-26, 2016, to test eDNA techniques during a massive coral spawning event. The team sailed on the Sanctuary vessel, the *R/V Manta*, and collected samples while diving to measure the diversity of genetic material swirling in the water above the coral during the spawning event. The team sought to determine whether eDNA can detect the diversity of spawning corals, sponges, and brittle stars as they released their sperm and eggs into the water during this annual massive reproduction event.

Mass coral spawning events are one of nature's most spectacular phenomena. They happen every year in the FGBNMS like clockwork, one week after the last full moon just before the end of summer. We were therefore ready for the reef to start spawning on the night of August 24, 2016, one hour after sunset. The team was uncertain whether this year's spawning event was going to happen on time due to an unprecedented die-off of corals, sponges, brittles stars, sea urchins, fish, and many other organisms in the eastern FGBNMS in June-July 2016. The causes of this die-off are still under investigation but we suspect this was the result of a massive and very quick offshore movement of coastal brackish water in late June and early July after weeks of heavy rainfall in Texas.

The FGBNMS diving team was led by Michelle Johnston, John Embesi, Ryan Eckert, Kelly Drinnen, and Dustin Picard. They went overboard right after the sunset to collect seawater bathing the corals before, during, and after the spawning event. They collected the samples in 1-liter sterile bags (Figures 12, 13, and 14).

Figure 12. USF graduate student Megan Hepner (left) and Michelle Johnston (right) collecting eDNA samples above a Montastraea spp. colony in August, 2016. Picture courtesy of the FGBNMS Research Staff.

Figure 13. Diver collecting eDNA samples above a Montastraea spp. colony using 1-liter sterile bag in August, 2016. Picture courtesy of the FGBNMS Research Staff.

Figure 14. *Pseudodiploria strigosa* during spawning on August 24, 2016. Picture courtesy of the FGBNMS Research Staff.

Samples were processed on the deck of the *R/V Manta*. The water was filtered through sterile filters, which were then stored frozen in liquid nitrogen for transportation to USF for eDNA analyses. Breitbart's group is currently extracting and sequencing DNA from this samples.

Sample Collections in the Monterey Bay National Marine Sanctuary (MBNMS)

From April 25 – June 7, 2016, the Stanford University (SU) group collected eDNA water samples on the annual NOAA Rockfish Cruise. The group collected over 300 filtered water samples (0.2 µm) across 7 stations at the depths of 10, 40, and 80m from Point Reyes to Piedras Blancas. The selected stations span beyond 276-mile length of the MBNMS, allowing us to observe and compare eDNA biodiversity across the largest NMS in the U.S. A subset of samples were processed to determine that all groups could use a standardized extraction protocol and therefore could share extracts for all targeted loci. Select samples from the 2016 Rockfish Cruise are currently being processed for sequencing the targeted 12S rRNA gene.

The MBNMS team (i.e. SU and MBARI) went on the annual CANON Cruise, a 9-day cruise (September 23 - October 1, 2016), to sample water for eDNA from 50 stations within MB. We sampled select stations and depths to expand our understanding of the MB ecosystem. The Bay environment was quite different from the conditions during our Rockfish Cruise in spring 2016, as there were algal blooms occurring throughout the Bay. The samples are contributing to the historical MB Time Series, a portion of which we are using as part of the MBON project.

From April 26 – May 5, 2017, Collin Closek, from the SU group, joined the annual Rockfish Cruise to collect a new set of eDNA water samples from some of the same locations as 2016. The samples were collected before trawls to compare eDNA richness to trawl catches. For the last 2 days of the sampling trip the Rockfish cruise rendezvoused with MBARI's Western Flyer and long-range autonomous underwater vehicle (LR-AUV) to simultaneously characterize the biodiversity within the water column (i.e. through eDNA collected via CTD, via LR-AUV, and fish trawls) (see Figure 15). Additionally, SU collected water samples for nutrients and filtered liquids from trawl catches.

Environmental DNA Method Optimization Efforts at the Center for Ocean Solutions

To optimize our methods for detecting Eukaryotes using eDNA, the eDNA team has compared multiple loci as well as the extraction methods to determine the limitations and preferences across the groups for robust taxa detection. Ryan Kelly and Collin Closek from the SU group along with other co-authors from NOAA compared detection of organisms across manual (i.e. visual counts) and three conserved eukaryotic molecular makers (16S, 18S, and COI). The Frontiers in Marine Science paper (Genetic and Manual Survey Methods Yield Different and Complementary Views of an Ecosystem) by Kelly et al., 2017, showed increased detection of a taxonomic group through multiple methods, however there were some taxa identified through manual counts that were not detected with the loci tested.

The eDNA team also conducted a methods comparison test to determine which filter type and extraction method yielded the most consistant results across the loci of interest. This comparison informed the standard method the eDNA team uses to collect and extract our eDNA water filter samples. The paper by Djurhuus et al. is currently under review.

Anchovies to Whales

Using historical samples from the MB Time Series, we examined the diversity of 12S rRNA sequences from samples collected during falls of 2008-2015. The sequences were compared to whale observation data collected by whale watching companies during the eight years. Anchovy DNA was quantified in the samples by a species-specific qPCR assay designed by Sassoubre et al., 2016. Patterns in these species of interest showed presence of specific organismal communities during certain years and an oscillation between sardine and anchovy in MB (Figure 14). The manuscript is in progress and is being prepared by Co-PI F. Chavez.

Microbes to Whales (m2w)

The biological communities in samples collected from both the Florida Keys (FK) and MB NMSs are being compared. Representative samples from the two Sanctuaries are being sequenced to identify taxa from different trophic levels by targeting the COI mtDNA, 16S, 12S, and 18S rRNA genes. We aim to gain a deeper understanding of the food webs and changes in the biodiversity in these environments by comparing the biodiversity trends across seasons and years. We are currently in the process of submitting our samples for sequencing. Collin Closek and Anni Djurhuus will be analyzing the resulting data and writing the corresponding manuscript.

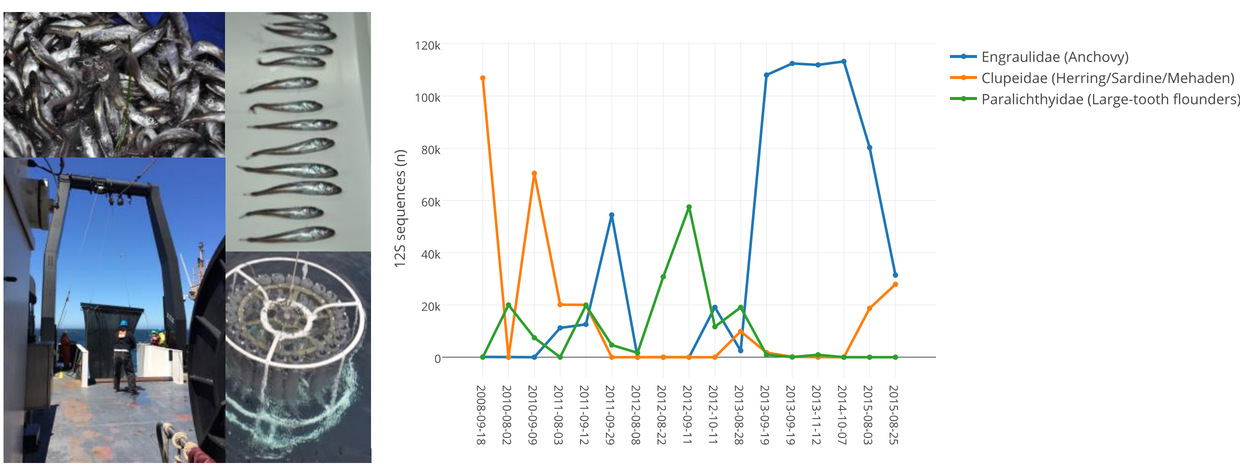


Figure 15. Left panel: 2017 Rockfish cruise photos. The team joined NOAA SWFSC's annual Juvenile Groundfish Cruise along the central California coast to compare eDNA samples collected to the diversity captured during trawl assessments. MBARI's RV Western Flyer joined the cruise on May 3 & 4, 2017. The rendezvous allowed for simultaneous collections of water using the LRAUV and CTD as well as fish counts from trawls. Right panel: The time series was dominated by sequences from Clupeidae (Sardine, Herring, Menhaden), Engraulidae (Anchovy), and Paralichthyidae (Large-tooth flounders). Sardine sequences dominated samples from MB during Fall 2008 and 2010, while Anchovy sequences dominated during Fall 2013 and 2014, which agrees with California fish catch numbers.

qPCR Assays for Detecting Organisms

We are designing quantitative PCR (qPCR) assays to detect DNA from specific species. The species targeted were selected based on their trophic importance in the MB food web by the entire MB MBON team. The SU group has designed qPCR assays to target Humpback Whale (*Megaptera novaeangliae*), Common Murre (*Uria aalge*), and Shortbelly Rockfish (*Sebastes jordani*). Samples of *U. aalge* tissue as well as tissue from other seabird species were acquired from the Ornithology Collection at Drexel University's Academy of Natural Sciences. *S. jordani* samples were obtained from the NOAA SWFSC Fisheries Ecology Division. We collected other fish species during the Rockfish Cruise in May 2016. *M. novaeangliae* and other marine mammal tissue samples were acquired from the Marine Mammal Center (MMC) in Sausalito, CA. DNA from all tissues samples have been extracted. qPCR optimization trials are currently in process for both the *U. aalge* and *M. novaeangliae* assays.

Results from the 2015 CANON Cruise

We finished analysis of the metabarcoding data from the 2015 CANON Cruise. The work was published in PLOS ONE.

Activities: Collin presented the Anchovies to Whales work as an oral presentation at Stanford's Postdoctoral Research Symposium and at the AGU Meeting in December 2016. Collin also presented this work as posters at Stanford's BIO-X Research Summer 2016 Symposium, Woods Institute's Young Environmental Scholars 2017 Conference, and at the American Society for Limonology and Oceanography 2017 Meeting.

We conduct eDNA calls twice per month. In addition, we had a 2-day MB-focused meeting across all the MBON teams in July 2016. Collin attended the SDG-14 MBON meeting in St. Petersburg, FL in March 2017 and continues to work with the biodiversity team to create a MBON SDG-14 product.

Summary of planned activities in the MBNMS for next year

1) We will complete the microbes to whales (*m2w*) sequencing and begin data analysis and writing.
2) We plan to work on the qPCR assays and hopefully finalize them and test them on eDNA extracts from the field.
3) Contribute to continued work on the anchovies to whales project with collaborators at MBARI.
4) Work with MBARI to harmonize results between autonomous vehicle and grab samples during the Rockfish Cruise. Work with MBARI to complete the autonomous vehicle assays.
5) Continue outreach with managers and policy makers on the use of eDNA for biomonitoring.

4. Seascapes and High-Resolution Satellite Remote Sensing Products

Sanctuaries MBON

Modern seascape classification merges lower trophic level ecology, geography, and ocean dynamics using observations that are updated regularly and that provide a historical context for reference against which to measure change (Figure 16).

Objective classification and validation of remotely-sensed dynamic seascapes using high resolution (1 km) satellite data has occurred at regional to local scales in temperate-upwelling (California) and tropical reef environments (Florida) as part of the US MBON pilot program. Seascapes provide a framework to assess and scale up patterns of biodiversity and effects of environmental change on pelagic community structure, ranging from microbes to fish, and provide indicators of habitat quality to inform National Marine Sanctuary Condition Reports. A goal of the MBON is to better understand the effects of climate and coastal ocean dynamics on spatiotemporal dynamics of marine species distributions in order to inform state and federal management. In concert with ship, buoy, and autonomous platform measurements, seascapes categories are being used as an objective extent to plan sampling, conduct rarefaction studies, inter-compare spatial and temporal patterns across trophic levels, test hypotheses of fisheries habitat affinities (e.g. Santora et al., 2012;

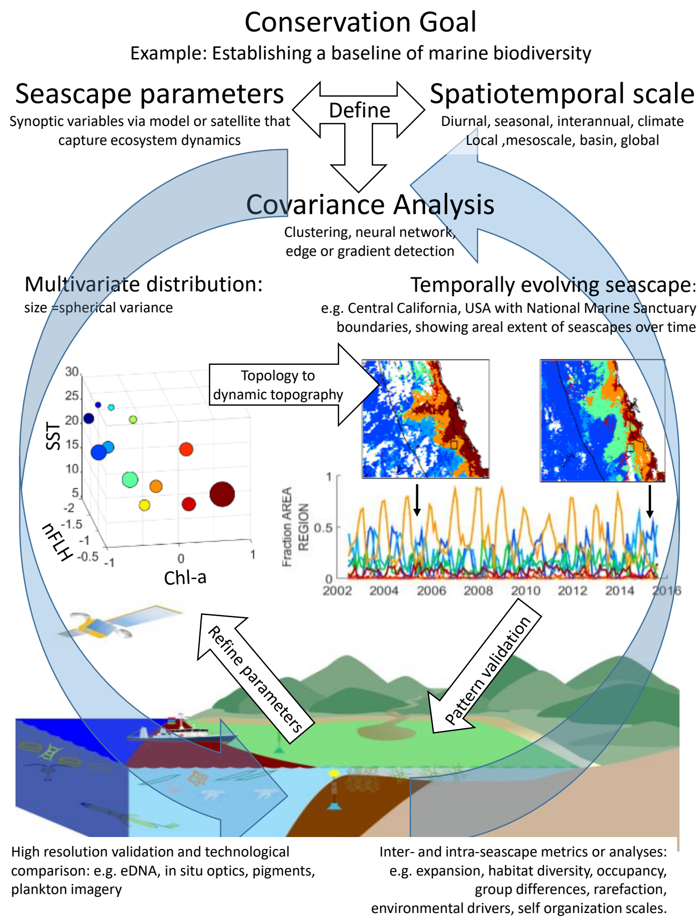


Figure 16. Seascapes as an observational and management tool. From Kavanaugh et al., 2016. Blue arrows denote the interplay between mechanistic hypotheses testing and analyses of emergent patterns. The conservation goal, spatiotemporal scale, and parameters of interest may determine whether synoptic time series of satellite remote sensing (2-D), assimilated marine ecosystem models (3-D) or both are used to define seascapes. Higher resolution in situ data can provide vertical data, but also higher resolution organismal information than that provided by remote sensing reflectances or model functional types. Finally, in addition to informing conservation (e.g. rarefaction, patch and boundary analyses), management (trends and oscillations of major habitats) inter- and intra-seascape analyses can inform basic scientific inquiry such as dominant environmental drivers, and scales of biological self-organization (e.g. through partial-mantel tests).

Kavanaugh et al., in prep; Figure 15), quantify seascape habitat diversity (Whitaker, 1977; Turner, 2005), and examine temporal shifts in habitat quality and availability within existing jurisdictional units.

MBON seascapes are classified at 1 and 4 km spatial resolution and at 8-day and monthly temporal resolution. The high spatial resolution is reserved for local regions; 4 km is used to characterize regional to basin scale patterns (such as the Pacific Arctic Sector, see Goal 3). The 8 day average allows for sufficient data density during periods where cloud cover obscures remote sensing of ocean color variables. For the 1 km case, Level-2 (L2) mapped files subsetted to a particular ROI will be ordered and then downloaded from the OBPG, which will allow greater flexibility to develop non-standard satellite products suited to the ecological regions of those areas. For example, the generalized ocean color inversion model (Werdell et al., 2013) will be used to approximate CDOM for the Arctic and thus provide more robust and universal inputs to regionally-tuned seascapes than default satellite products previously designed for use in the open ocean. Additional products include phytoplankton functional types, derived from empirical relationships to remote sensing reflectances (e.g. diatoms in Antarctic coastal waters; Kavanaugh et al., 2015) or from theoretical inversions to absorbance and scattering (e.g. Mouw and Yoder, 2010; Kostadinov et al., 2009). These algorithms provide information on community structure and habitat quality beyond that of chl-a. The raw data are processed and stored at University of South Florida Institute for Marine Remote Sensing.

Cross MBON efforts (X-MBON)

Multivariate satellite seascapes allow us to document change in pelagic habitat type and water quality within coastal national exclusive economic zone boundaries (EEZs), provide oceanographic context for long term ecological monitoring projects (e.g. the NSF-funded Long Term Ecological Research program) and internationally over the high seas and Ecological or Biologically Significant Marine Areas. The latter are regions identified by the Convention on Biological Diversity as areas in open-ocean waters and deep sea habitats in need of protection based on the following criteria: uniqueness and rarity, special importance for life history stages, importance for threatened, endangered or declining species or habitats, vulnerability, productivity, diversity, and naturalness, the latter indicating the degree of human impact.

Some X-MBON efforts led by the Sanctuaries MBON are further described below.

The Arctic MBON (AmBON)

The Arctic is experiencing dramatic change, with declining seasonal sea ice, earlier sea ice retreat, increased ocean temperatures and freshening of the water column with increased glacial and permafrost melt (Woodgate et al., 2012; Stroeve et al., 2014; Frey et al., 2014, 2015; Wood et al., 2015).These environmental shifts result in significant alteration of marine structure and function, driving shifts in marine species composition, phenology, and carbon cycling (Grebmeier et al., 2006b; Bluhm and Gradinger, 2008; Nelson et al., 2014). An important co-factor in this trend is the advection of Pacific water into the Arctic (Carmack and Wassmann, 2006) from the northern Bering Sea to the Chukchi Sea (Stigebrandt, 1984; Kinder et al., 1986; Danielson et al., 2014). Additional heat, nutrients, organic carbon and organisms are advected through the Bering Sea into the Chukchi Sea, contributing substantially to seasonal local production (Coachman et al., 1975; Sambrotto et al., 1984; Walsh et al., 1989). Following several inflowing pathways driven by bathymetry, the inflow of Bering Sea water leaves a signature on seasonal sea ice retreat as unique water mass characteristics within the melting ice edge (Paquette and Bourke, 1981; Wood et al., 2015). The importance of the Arctic Ocean to global climate and ecosystem processes, and the speed at which climate changes are already occurring in the Arctic, elevate the urgency for coordinated observations of Arctic marine biodiversity.

The Arctic Marine Biodiversity Network or AMBON involves an experienced team of multi-institutional and multi-sector partners already active in a variety of Arctic biodiversity observing programs. However, until recently, the AMBON has not had an integrated satellite seascape observing component. This partnership within AMBON and across the US-MBON program will allow us to better coordinate, sustain, and synthesize all efforts, and make data available to a broad audience of users and stakeholders, from local to the pan-Arctic region up to the global system. Effective data management, integration and dissemination will provide critical information on the status of Arctic ecosystem health and resilience to decision makers and local, regional and global communities.

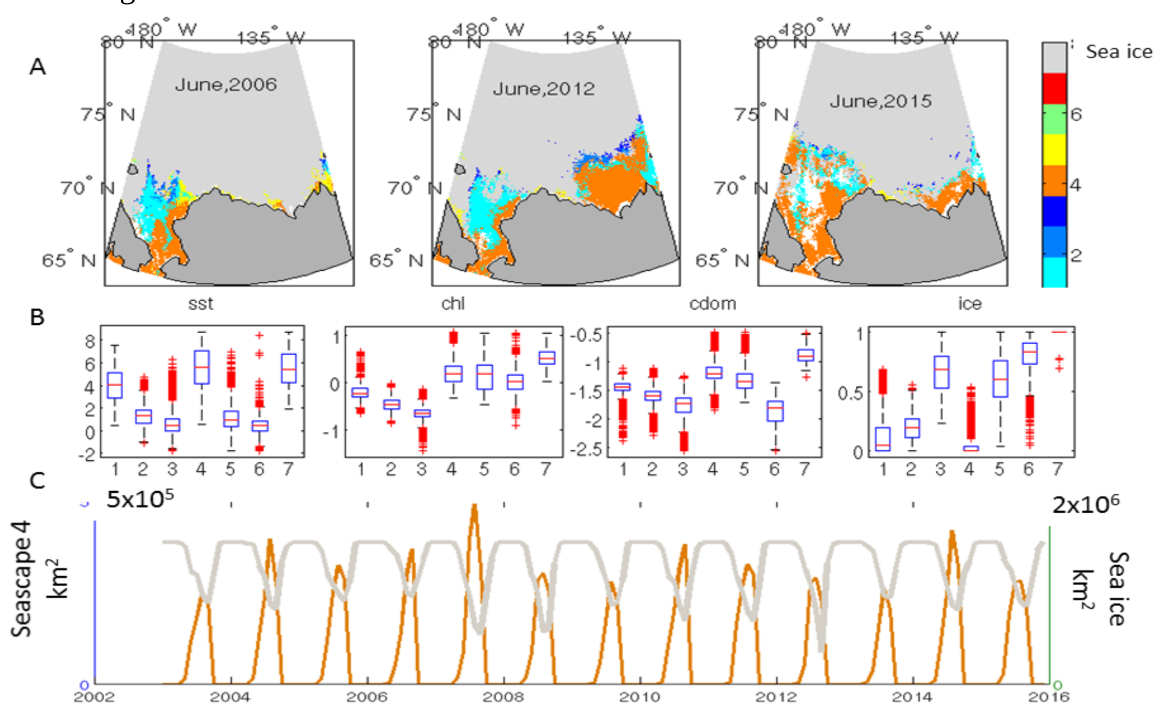


Figure 17. Preliminary results showing feasibility for satellite derived seascape classification for the Arctic. A. June seascapes for three different years classified from sea-ice concentration, CDOM, Chl-a, nflh, and SST. Mean qualities of classified seascapes showing relative influence of sea ice and ocean color in open water (1-3, blues in A) ice edge (5 and 6) and high productive shelf seascape (4, 7) C. Time Series of high Chl-a shelf seascape (orange) extent and ice coverage within the domain .

An important feature of the AMBON is the focus on benthic biodiversity. Benthic macrofaunal communities on the shallow continental shelves of the Pacific Arctic accumulate high biomass in response to high levels of seasonal export of pelagic production that is either being advected into the system from upstream primary production or produced locally and exported to the underlying sediments directly (Grebmeier et al., 2006a; Nelson et al., 2014). The interaction between circulation features and local production inputs have resulted in persistent benthic hotspots. These four hotspots are also associated with high chlorophyll a (chl a) content in the water column (Springer et al., 1996; Hill and Cota, 2005; Lee et al., 2007; Brown et al., 2011; Cooper et al., 2013), and thus are visible from satellite ocean color.

We have hypothesized that changes in Chl-a, dissolved organic matter and temperature will be evident from the spatial patterns of satellite derived seascapes. Seascapes will provide context for the Chukchi Sea AMBON and DBO programs, and the identification and geographic analyses of dynamic surface water masses will contribute to understanding the relative influence of regional and local inputs to both pelagic and benthic hot spots. Because of the unique nature of the environment, a seascape classification scheme will also consider the dynamics of sea ice and create indices (e.g. Figure 17-C) that are of use to end users interested in pelagic-benthic relationships. Indices will include variability of seascape habitat extent and persistence of high productivity seascapes over benthic hotspots.

Data to validate remotely sensed seascapes will come from the AMBON for the Chukchi Shelf and the Distributed Biological Observatory (DBO) for the northern Bering, Chukchi, and Beaufort Seas.

Santa Barbara Channel Long Term Environmental Research

PI Kavanaugh is working with Investigator Dan Otis at University of South Florida and PI Robert Miller of the Santa Barbara Channel LTER MBON to develop seascape prototypes for the Santa Barbara Bight. These maps will form the basis of neutral models of larval dispersal as well as environmental indices for kelp forests. Kavanaugh is classifying seascapes based on a regional classification model of the California Current to provide a means of comparison between US West Coast MBONs.

U.N. Sustainable Development Goal 14 Biodiversity Product

The Group on Earth Observations (GEO, https://www.earthobservations.org) Thematic Marine Biodiversity Observing Network (MBON) supports the operational monitoring of marine biodiversity, defined broadly as the variety of life at the gene, species and ecosystem levels. Understanding how biodiversity changes across these levels provides insight to ecosystem resiliency or vulnerability to global change, and their capacity to maintain the vital ecosystem services on which humans depend. The systematic observation of life in the global ocean requires both standardized in-situ (field observations) and remote sensing methods, and a means for stakeholders to compare status and trends between locations.

Biogeographic studies have sought to characterize variation of life forms and ecosystems at different space and time scales for some time (Dasmann, 1968; Longhurst, 1998; Briggs and

Bowen, 2012). Recently, a new map of global ecological land units (ELUs) was developed from an integration of climate, landform, lithology, and land cover (Sayre et al., 2014). The mapping effort was commission by GEO, was data-driven, and based on the physical environmental controls which are understood to be the primary control of species distributions (Bailey, 2009). Partnering with ESRI and several other partners (including NatureServe, University of Auckland, GRID-Arendal, and WHOI), the USGS now has a similar environmental stratification approach for extending the global ecosystems map into the oceans through the delineation of global ecological marine units (EMUs; Sayre et al., in accepted). The EMU process recognizes that marine ecosystems are not two dimensional, but operate as both 2D (e.g. sea surface and seafloor) and 3D (e.g. water column) entities (e.g., Li and Gold, 2004; Wright et al., 2007).

The EMU project classified distinct volumetric regions using data from the World Ocean Atlas (Locarmini et al., 2013; Garcia et al., 2013; Garcia et al., 2014). Temperature, salinity, dissolved oxygen, nitrate, silicate, and phosphate multi-year climatological means populate a ¼ degree ocean mesh horizontally that is extended over 102 depth zones in the vertical direction. A k-means statistical clustering of the six physicochemical variables listed above at over 52 million latitude x longitude x depth zone locations, identified 37 physically distinct, relatively homogenous, volumetric regions in the water column (EMUs). Temporally the EMUs represent a 57 year climatological mean of water mass location. A paper highlighting the results and process is in review (Sayre et al., accepted) and an informational web site, data portal, and interactive tool are available to stakeholders through ESRI (http://www.esri.com/ecological-marine-units). Ongoing efforts by the EMU project seek to extend the multi-year climatological classification of physical and chemical data to a seasonal climatological classification.

The Sanctuaries MBON team is working with the EMU team to link global synoptic seascapes.

Global synoptic seascapes

Over the last decade, methods to study spatiotemporal patterns of biological variables using satellite-based remote sensing have improved significantly (Saraceno et al., 2006; Platt and Sathyendranath, 2008; Mouw and Yoder, 2010; Mouw et al., 2012; Reygondeau et al., 2013). The dynamic 'seascape' concept was introduced as a means of categorizing the complex, multi-scale patterns of open ocean ecosystems into an organized, hierarchical biogeographical framework (Kavanaugh 2014; 2016). Satellite seascapes incorporate the complexity of biophysical interactions and the circulation of the fluid environment. The method is robust to nonlinear responses of marine taxa to physical forcing, which are prevalent at cellular (Jassby and Platt, 1976) to community levels of organization (Steele and Henderson, 1992; Litzow and Ciannelli, 2007). The use of synoptic satellite data allows for seascapes to be objectively defined at multiple, nested scales (Kavanaugh et al., 2014). For open ocean systems, this objective method is more efficient than more subjective static biogeographic assessments (Longhurst, 1998). Satellite derived seascapes provide the necessary context for understanding biodiversity across the diverse coastal habitats represented by the US-MBON pilot program.

Seascapes are classified within the MBON using a suite of synoptic time series including satellite and modeled variables. These variables include satellite-derived SST, PAR, chl-a, normalized fluorescence line height (a metric of phytoplankton nutrient status), dynamic height (SSH), and modeled salinity and mixed layer depth. Most variables are direct satellite based measurements; some however (e.g. salinity and mixed layer depth) are modeled (Estimating the Circulation and Climate of the Ocean: ECCO v.2). Salinity was available from the Aquarius satellite mission for a short period, however the spatial footprint (1 degree) is too large for local application.

Horizontal Co-Location with Annual and Seasonal Ecological Marine Units (EMUs)

The WOA data upon which the EMUS are based have several native temporal resolutions (seasonal, annual, and decadal) that we plan to exploit as a next step. We now have we have established a framework for constructing temporally-sequenced EMU distribution maps for seasonal, annual, and decadal characterizations. We are developing the present 3D framework to show changes at each location over time, to facilitate the horizontal co-location of annual and seasonal EMUs with surface seascapes.

Water Column Variability Underlying Global Seascapes

Current vertical distribution of the EMUs through the water column shows cluster either slowly disappearing with depth, with no clear-cut horizontal boundary (Sayre et al., 2017). A comparison between the boundaries of realms and ecosystems in the upper water column underlying global seascapes should indicate what environmental gradients have most strongly influenced the evolution of biodiversity by being barriers to species dispersal. This in turn should inform as to what volumetric regions (realms and ecosystems) may be the most valuable for environmental management because of similar environmental conditions and species composition (Costello et al., in press). Fidelity of EMUs (discrete sense) and coherence of variability (continuous sense) are being compared to surface signature of distinct seascapes and their boundaries. Multivariate analysis of variance and clustering are being used to determine where or if surface seascapes can determine unique properties in the underlying water column.

Seascapes and Large Marine Ecosystems

A manuscript entitled *Megaregions Among the Large Marine Ecosystems of the Americas* was published in 2017 by a team of Sanctuary MBON co-authors (*Muller-Karger, F., Digna Rueda-Roa, Francisco Chavez, Maria Kavanaugh, and Mitchell A. Roffer. 2017. Megaregions Among the Large Marine Ecosystems of the Americas. Environmental Development. http://dx.doi.org/10.1016/j.envdev.2017.01.005.). With this study, we found:

1) Three megaregions emerged when considering similarities in species composition of fisheries landings, fisheries-derived diversity indices, and characteristic environmental conditions among LMEs. Specifically, these include (A) the South Brazil Shelf, East Brazil Shelf, and North Brazil Shelf LMEs, (B) the Gulf of Mexico and Southeast U.S. Continental Shelf LMEs, and (C) the Northeast U.S. Continental Shelf, Scotian Shelf, and Newfoundland-Labrador Shelf LMEs.

2) No apparent megaregions emerged for the Pacific Ocean. While there were some shared species assemblages between the California Current and the Gulf of Alaska, the Gulf of California, and the Pacific Central-American Coastal LMEs, these showed different average

environmental conditions and fishery-derived diversity indices, so they don't form one ecological megaregion.

3) Between 1982 and 2010, seven different LMEs diversified their fisheries (Pacific Central-America Coastal, Patagonian Shelf, South Brazil Shelf, East Brazil Shelf, North Brazil Shelf, Southeast U.S. Continental Shelf, and Newfoundland-Labrador Shelf). Three LMEs showed increasingly less diversified fisheries, namely the California Current, the Northeast U.S. Continental Shelf, and the Caribbean Sea LMEs. The causes for either an increase or decrease in the diversification of the fisheries are numerous, and can range from large-scale environmental forcing like warming of the Atlantic Ocean to focused conservation efforts such as in the California Current LME.

Current and Future Multidisciplinary Remote Sensing Work : Sustainable Marine Biogeographic Framework for Conservation and Management: IOOS, ESRI and COVERAGE

PI Kavanaugh has been involved in several activities geared toward sustainability of MBON-related products. Satellite seascape processing and products are in the process of being automated so that maps can be provided to stakeholders in real time. At the local scale (e.g. within the existing US-MBON projects), IOOS data portals are used to store seascape output. On the global scale we are collaborating with NASA Jet Propulsion Lab and ESRI to trace a path to the sustained generation of MBON satellite products. Initially, collaborators at JPL will serve as advisors in the capacity of the Physical Oceanography Distributed Active Archive Center (PO-DAAC). The PO-DAAC is a key source of multiscale satellite data to classify seascapes. However, our colleagues at JPL are also initiating the COVERAGE program (CEOS Ocean Variables Enabling Research and Applications for GEO). COVERAGE is intended to integrate observations from four separate international oceanographic satellite constellations in near real-time.

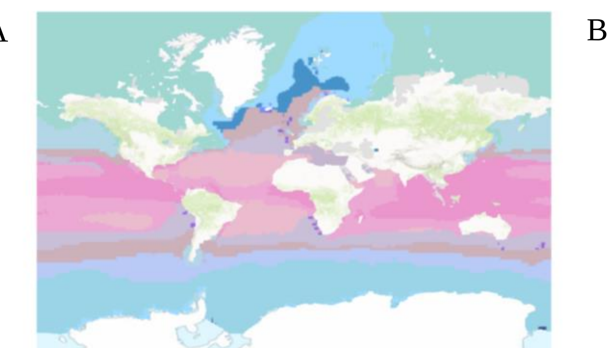
Kavanaugh is also working closely with ESRI, who were responsible for building the original 3-D point mesh framework out of over 52M points extracted from the World Ocean Atlas, for performing the statistical clustering, creating the project web site and data portal, and for producing a range of data products (e.g., open access downloads of the 3D point mesh and EMU clusters at the surface, bottom, and within the water column, as well as 2D and 3D web apps for exploration of the EMUs and the original World Ocean Atlas data, data dictionaries, and data sheets) (Figure 18). ESRI will continue to host these data products as well as additional data products resulting from this project and new tools for 3D web viewing, 3D cross sections, and analysis tools as they are developed (e.g., compare multiple locations, multidimenstional range slider, and 3D kriging). ESRI will collaborate with the JPL COVERAGE group in hosting data to ensure that all data and map products are accessible in multiple formats and regularly updated. ESRI will also continue to take the lead in adding more associative attributes to the EMUs from other globally available resources, including Ocean Biogeographic Information System (OBIS) species records at the surface and at depth where available, and the direction and velocity of currents. All the aforementioned work will be provided as in-kind support at no cost to the project due to the alignment of this project with ESRI 's current R&D initiative in ocean GIS (ESRI invests ~31% of its revenues back into R&D). ESRI Chief Scientist Dawn Wright will continue to supervise this work at ESRI in concert with ESRI product development teams in content and spatial statistics.

The NOAA NESDIS CoastWatch program (Paul DiGiacomo) has expressed a commitment to engage in discussions to host both U.S. regional seascape products at high spatial resolution (1 km) and global products at reduced resolution (possibly 4 or 9 km). These discussions are now underway as of June 2017.

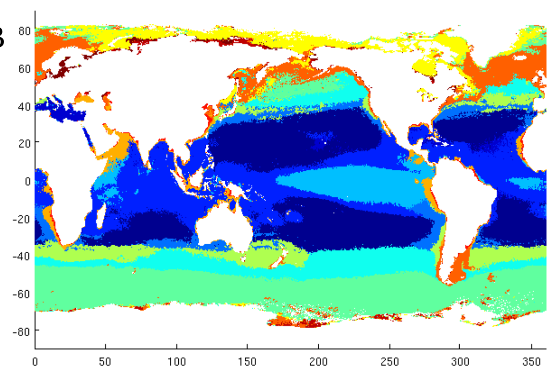
MBON and SDG-14 Satellite Products

Satellite products for the MBON and SDG-14 projects are created at three different spatial resolutions, 1-km, 9-km and 0.25 degree. Global ocean color products are binned to monthly intervals, while regional 1-km products are binned to 8-day time intervals (Table 2 and 3). All ocean color products utilize data from the Moderate Resolution Imaging Spectroradiometer (MODIS-Aqua) satellite sensor and are obtained from the Ocean Biology Processing Group (OBPG) at NASA's Goddard Space Flight Center. Satellite image files are downloaded via ftp to USF servers and subsequently processed at USF. Output products are stored and distributed to MBON and SDG-14 team members via ftp from USF servers.

A



B



C

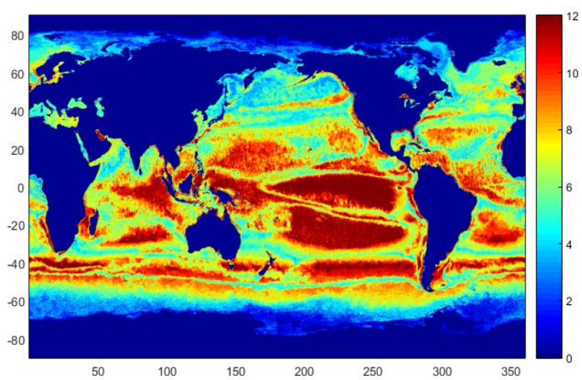


Figure 18. Intercomparison of surface classification schemes showing surface EMUs (A), dominant seascape classified from SST, Chl-a and nflh, the latter giving a sense of nutrient stress (B), and number of seascapes represented in a given pixel (C). Note the similarity in boundaries between A and B and the importance of seasonal boundary shifts that C represents.

In Year 3, we have added regions of interest (ROI) to cover the Santa Barbara Channel and Flower Garden Banks. We have also added a full set of products that cover the entire globe as part of the SDG-14 project. Information on ROI, spatial resolution and parameters of satellite products are given in the two tables below. To establish an extended SST record, we have also added a Global High Resolution Sea Surface Temperature (GHRSST) product that extends back to 1982. This product incorporates data from both satellite and instrument measurements and has a spatial resolution of 0.25 degree. Daily image files are obtained from the Jet Propulsion Laboratory (JPL) Physical Oceanography Distributed Active Archive Center (PODAAC) and binned temporally to monthly intervals to match the rest of our global products.

Our plans for Year 4 include further automation of our processing scheme and incorporation of the final seascape generation to make the overall process as streamlined as possible while requiring the least amount of day to day input by USF personnel. We also plan to add other ROI as needed to facilitate seascape analysis, particularly with potential international partners.

Table 2. Regions of interest (ROI) for MBON and SDG-14 satellite products

Region	ID	Resolution	Latitude	Longitude
Global	GLOB	9-km, 0.25 Deg.	90°S – 90°N	180°W – 180°E
Chukchi Sea	CHUK	1-km	64°N – 73°N	180°W – 160°W
Florida Keys	FK	1-km	23°N – 26°N	84°W – 79°W
Flower Garden Banks	FGB	1-km	24°N – 31°N	98°W – 88°W
Gulf of Mexico	GOM	1-km	18°N – 31°N	98°W – 79°W
Monterey Bay	MONB	1-km	34°N – 40°N	128°W – 120°W
Santa Barbara Channel	SBA	1-km	31°N – 35°N	121°W – 117°W

Table 3. Output products being generated

Parameter	Algorithm	ID Code	Units	Reference
Chlor_a	CI (default)	chlor_a	mg/m^3	Hu et al. (2012)
Chlor_a	ocx	chl_ocx	mg/m^3	O'Reilly et al. (2000)
Chlor_a	Statistical	chl_sw	mg/m^3	Wattelez et al. (2016)
CDOM	GIOP	adg_443	m^{-1}	Werdell et al. (2013)
Backscatter slope	GIOP	bbp_s_44	$nm^{-1}m^{-1}$	Werdell et al. (2013)
PAR	Default	par	$einstein/m^2/day$	Frouin et al. (1989)
NFLH	Default	nflh	$W/m^2/\mu m^2/sr$	Behrenfeld et al. (2009)
SST – Day	Long-wave	sstd	Deg. C	N/A
SST – Night	Short-wave	sstn	Deg. C	N/A

5. ***GEO BON MBON and Other International Efforts***

The leadership of the Sanctuaries MBON of the MBON task team of the GEO Bon Working Group 5, jointly with Mark Costello (U. Auckland) and Isabel Sousa Pinto (U. Porto), led to the formal establishment of the Thematic MBON under GEO BON in summer 2016. The MBON group has been meeting monthly over webinars. The team has continued to develop a vision of *"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"* to:

- promote the establishment of best practices for global biodiversity observations;

- improve the collection of harmonized data, developing data standards and methodologies for data management and dissemination;
- facilitate data sharing without compromising national concerns;
- integrate biodiversity information with physical and chemical data over time (status and trends); and
- generate the products needed for informed management of the global ocean.

The GEO MBON seeks to assist in identifying change in the diversity of life in the ocean as an indicator of ecosystem change, in support of critical management decisions by coastal and ocean resource managers. Changes in communities, species and habitats are indicators of environmental change relevant to ecosystem services and the well-being of humanity. The MBON helps the integration and coordination of national and international programs for marine biodiversity observation by providing improved access to data, tools, and expertise. The MBON will contribute to the development and measurement of marine Essential Biodiversity Variables, and contribute methods and tools for a Marine BON in a Box.

The GEO MBON grows out of the priority activities of the GEO BON WG5. It includes and builds on the Global Marine Ecosystem Mapping effort (Sayre et al., 2015). It incorporates the biodiversity priorities of various GEO initiatives, including Blue Planet and AmeriGEOSS, and coordinates with IOC/UNESCO (GOOS and I-OBIS), and other national and international groups to serve the broadest possible community.

The MBON seeks to help nations and regions to improve conservation planning and environmental impact mitigation, serve the scientific community, and satisfy commitments to the Convention of Biological Diversity (CBD) and the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES).

GEO MBON and SDG14 pilot product developments were presented at many meetings and workshops, including:

- The GEO Marine Biodiversity Observation Network (MBON) Workshop, July 6-7, 2016, Leipzig, Germany.
- GEO MBON Pole-to-Pole of the Americas Workshop, September 26-30, 2016, Puerto Morelos, México.
- GEO Ministerial Meeting / November 2016 / St. Petersburg, Russia.
- CBD Side Event: 3rd Science for Biodiversity Forum - Mainstreaming Biodiversity for well-being: Contributions form Science. December 1-2, 2016, Cancun, Mexico.
- Future Earth Meeting – December 2016, Kiel, Germany
- SDG14 Demonstration Product, March 15-17, 2017, St Petersburg, FL.
- Blue Planet, May 2017, Washington DC.

An important international initiative led by MBON was the signing of a collaboration agreement between MBON, the Global Ocean Observing System (GOOS), and the Ocean Biogeographic Information System (OBIS). The goal of signing the agreement is to:

- foster a partnership with the Intergovernmental Oceanographic Commission (IOC) to define Essential Biodiversity Variables (EOV) under the GOOS Essential Ocean Variables (EOV);
- engage OBIS to expand the community that uses specific standards to use and share biodiversity data.

MBON will continue to be fully engaged in the GOOS Bio-Eco panel to promote and implement the amalgamation of the EOV and EBV.

6. Highlights from PR and E&O

Newsletters and websites: Reynolds and Montes edited/produced four issues of the Sanctuaries MBON Update in 2016-2017 to inform partners and external collaborators about current research activities. The marinbon.org website, launched in Feb 2016, was revised to focus on U.S. MBON strategies and initiatives. The Sanctuaries MBON project site http://www.sanctuaries.marinebon.org was launched June 22.

Presentations and Videos: Dr. Kathy Sullivan (NOAA Administrator and Under Secretary of Commerce for Oceans and Atmosphere through 2016) gave a 20 minute presentation at the IUCN World Conservation Congress (September, 2016) about programs and new tools to monitor marine biodiversity. The Sanctuaries MBON team supported her communications staff with images of MBON projects including: AUVs, eDNA, integrated datasets/interactive portals; seascapes and collaboration with USGS on the EMUs tool. A clip from a video created by Ben Best to showcase the MBON portal was included in Dr. Sullivan's presentation. The full three-minute video is on several MBON websites. Reynolds is working with Sanctuaries staff and AOML researchers to create short videos, similar to the "Earth is Blue" series. Interviews were conducted in March 2016, b-roll collected in April-June. Editing will be completed in the summer. Release is planned for Sept 2017.

Topic-focused outreach: MBON researchers and the communications staff at the Florida Fish and Wildlife Conservation Commission/ Fish and Wildlife Research Institute (FWCC/FWRI) and Stanford University Center for Ocean Solutions (COS) implemented activities on environmental DNA. In Nov. 2016, the FWCC/FWRI created a visually engaging story map to inform managers and stakeholders about the use of environmental DNA sampling methods and its importance to FWRI's Harmful Algal Blooms (HAB) research program. The story map focuses on the use of the genomics tools/methods developed by MBON, as part of FWCC/FWRI monitoring and event response. In Dec 2016, MBON eDNA was featured on the BBC World Service radio. Collin Closek (COS) was interviewed about the work he and Alexandria Boehm conducted on vertebrate fishery assessments with metabarcoding eDNA. In Feb. 2016, Closek presented MBON eDNA research and the Monterey Bay, CA Fall historical time series at Stanford's Young Environmental Scholars (YES) Conference. In June 2017, Kristen Weiss (COS) created "Solutions," a new video series, one of which featured MBON eDNA to create awareness of early career scientists and innovative research.

Youth Outreach and Education: In St. Petersburg, Florida, GCOOS O/E lead Chris Simoniello conducted activities at three school science festivals and the 2016 St. Petersburg Science

Festival, which reaches more than 200 children. Simonello combined resources from MBON and a grant from the NOAA Climate Stewards Program to develop 12 hands-on STEM lessons aligned to Florida education standards for grades 1-8. She taught four lessons to 60 gifted elementary students. Lesson were adapted and taught by teachers to more than 640 elementary students and high school students in schools which have a majority of students who qualify for Title 1 status and 60% are African American. In August 2016, Simonello, Montes and Reynolds led a small group discussion with teachers at the Hillsborough Public High School district-wide Science Educator Professional Development Day.

MBON webinars: F. Muller-Karger convenes the following separate monthly MBON webinars:

- Sanctuary MBON: a monthly call to review Sancuary requirements and provide project updates to Sanctuary personnel.
- X-MBON coordination: Muller-Karger has tried to promote a dialogue between the various U.S. MBON projects (AK MBON, SBC MBON, Sanctuaries MBON and the Smithsonian Institution's Tennenbaum Marine Observatory)
- Global MBON: a monthly webinar with the international GEO BON MBON community has highlighted a variety of things such as the GEO Wetlands concept, specific international programs focused on a particular region, links between MBON, GOOS, and OBIS, various scientific paper discussions, and concepts for BON in a Box, among many other topics.

7. ***Budget Expenditures:***

At the University of South Florida, we continue to support one Master's graduate student (M. Hepner) and Research Associate E. Montes. PI Muller-Karger has 1 month summer salary charged to this grant. Other students, postdocs and researchers are supported through the several project subcontracts.

Budget expenditures are on track. There are no changes to the budget for Year 4.

8. ***Peer-Reviewed Publications and Other Products***

Andruszkiewicz, E. A., H. A. Starks, F. P. Chavez, L. M. Sassoubre, B. A. Block, A. B. Boehm. 2017. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. PLOS ONE 12(4): e0176343.

Anni Djurhuus, Collin J. Closek, Jesse Port, Hilary Starks, Kevan Yamahara, Ofelia Romero, Kristine Walz, Dawn Goldsmith, Reiko Michisaki, Mya Breitbart, Alexandria A. Boehm, Francisco P. Chavez. Standardizing filter type and extraction method for marine biodiversity monitoring using environmental DNA. (in review).

Biller, S.J., L.D. McDaniel, M. Breitbart, E. Rogers, J.H. Paul, S.W. Chisholm (2017). Membrane vesicles in seawater: heterogeneous DNA content and implications for viral abundance estimates. ISME Journal. 11: 394-404.

Kavanaugh, M.T., Rheuban, J., Luis, K. and S. Doney, Thirty-four years of benthic warming on the New England Shelf: patterns, drivers and ecological consequences. Submitted JGR Oceans

Kavanaugh, M.T., Oliver, M., Chavez, F., Letelier, R.M., Muller Karger, F. and Doney, S.C. 2016. Quo Vadimus: Seascapes as a new vernacular for ocean monitoring, management and conservation. ICES Journal of Marine Science 73 (7), 1839-1850

Mackey, K.M., Kavanaugh, M.T. Chen, Y., Liu, F., Glover, D., Paytan, A. Atmospheric and fluvial nutrients fuel harmful algal blooms in the East China Sea. 2017. Front. Mar. Sci. 4:2. doi: 10.3389/fmars.2017.00002

Montes, E., M. T. Kavanaugh, J. P. Cannizzaro, D. Otis, A. Djurhuus, L. Visser, K. Hubbard, F. Muller-Karger (*in prep*). Validation of dynamic satellite seascapes classification for understanding marine biogeography and scaling ocean biodiversity observations in the Florida Keys National Marine Sanctuary. Marine Ecology Progress Series.

Muller-Karger, F.E., Rueda-Roa, D., Chavez, F.P., Kavanaugh, M.T. and Roffer, M.A., 2017. Megaregions among the large marine ecosystems of the Americas. 2017. Environmental Development. http://dx.doi.org/10.1016/j.envdev.2017.01.005 In press

Rheuban, J., Kavanaugh, M.T., and S. Doney, Implications of future Northwest Atlantic bottom temperatures on the American Lobster (Homerus americanus) fishery. Submitted JGR Oceans

Ryan P. Kelly, Collin J. Closek, James L. O'Donnell, James E. Kralj, Andrew O. Shelton, Jameal F. Samhouri (2017). Genetic and Manual Survey Methods Yield Different and Complementary Views of an Ecosystem. Frontiers in Marine Science, 3:283

Sayre, R. , D. J. Wright, S. P. Breyer, K. A. Butler, K. Van Graafeiland, M. J. Costello, P. T. Harris, K. L. Goodin, J. M. Guinotte, Z. Basher, M. T. Kavanaugh, P. N. Halpin, M. E. Monaco, N. A. Cressie, P. Aniello, C. E. Frye, and D. Stephens. A three-dimensional mapping of the ocean based on environmental data. In press. Oceanography

Sassoubre, L. M., K. M. Yamahara, L. Gardner, B. Block, A. B. Boehm. 2016. Environmental DNA (eDNA) shedding and decay rates of three marine fish. Environmental Science & Technology, 50(19), 10456-10464

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