Coral Disease Workshop

Nova Southeastern University Oceanographic Center

Hosted by
Florida Department of Environmental Protection (DEP) and Florida Fish and Wildlife Conservation Commission (FWC)

Priority Sampling Plan Workshop (Part 1)
November 7, 2017

Facilitator: Karen Bohnsack

Attendees: Greta Aeby (University of Hawaii), Nick Alcaraz (FWC FWRI), Karen Bohnsack (DEP FCO), David Cox (DEP CRCP), Ananda Ellis (FWC FWRI), Dave Gilliam (Nova Southeastern University), Lindsay Huebner (FWC FWRI), John Hunt (FWC FWRI), Kristi Kerrigan (DEP CRCP), Yasu Kiryu (FWC FWRI), Vladimir Kosmynin (DEP DWRM), Jan Landsberg (FWC FWRI), Lauri MacLaughlin (NOAA ONMS SEGOM), Maurizio Martinelli (NOAA Coral Management Fellowship), Margaret Miller (SECORE), Erinn Muller (Mote Marine Lab), Karen Neely (Florida Keys Community College), Francisco Pagan (DEP CRCP), Valerie Paul (Smithsonian Institution), Rob Ruzicka (FWC FWRI), Stephanie Schopmeyer (FWC FWRI), Jennifer Stein (The Nature Conservancy), Joshua Voss (Florida Atlantic University), Joanna Walczak (DEP FCO), Brian Walker (Nova Southeastern University), Amber Whittle (FWC FWRI), Cheryl Woodley (NOAA NCCOS).

Welcome

- Joanna Walczak welcomes everyone to the 2nd workshop. She reviewed what happened on day one and the goals of the next two days.
- Karen Bohnsack highlighted the main objectives of the upcoming discussions as (1) creating a Florida Reef Tract-wide coral disease priority sampling plan to include both single event biological collections and fixed station assessment and (2) developing a plan for how existing and future samples should be analyzed across all disciplines, and identifying labs/personnel with the expertise to complete those analyses.

Presentation: **Overview of Florida Reef Tract Coral Disease Outbreak (Rob Ruzicka, FWC FWRI)** *Results in this study are ongoing, please contact Rob.Ruzicka@MyFWC.com for updates.

- The current disease outbreak along the FRT originated in Miami in 2014. It spread rapidly north and slowly south and has since spread southbound throughout the Upper Keys and northbound into Martin County. As of July 2017, the southern boundary was Tennessee Reef.
- Signs of disease were present at Tennessee Reef before Hurricane Irma and have worsened since the storm.
- The disease is affecting primary reef building corals, which can cause full reef mortality. A full list of affected species was presented from CREMP and SECREMP data:
- A video of Hens and Chicken Reef shows ~50-60% infection of susceptible species.

Presentation: Coral Disease Sampling Effort (Lindsay Huebner, FWC FWRI) *Results in this study are ongoing, please contact Lindsay.Huebner@MyFWC.com for updates.

- The FWC FWRI sampling effort includes collection of core sets for molecular analysis and histology, samples for TEM, and mucus samples.
- Two cores and a mucus sample were collected from apparently healthy corals, four cores and a mucus sample were collected from diseased corals. There were also reference sample sites for healthy corals.
- Grecian Rocks was sampled in July 2016. Four corals that were predominantly affected and provided a large enough sample size were selected for sampling:
    - *Montastraea cavernosa*, *Diploria labyrinthiformis*, *Siderastrea siderea*, and *Colpophyllia natans*
- Broward County was sampled in November 2016. Three corals were selected for sampling:
    - *Orbicella faveolata*, *Montastraea cavernosa*, *Siderastrea siderea*.
- Martin County was selected as a reference site in April 2017, before it was diseased. Four corals were taken from the northern reef:
    - *Diploria clivosa*, *Montastraea cavernosa*, *Porites astreoides*, and *Siderastrea siderea*

  All coral species sampled from the diseased reefs were also sampled from reference sites in Martin County and the middle Keys.

Presentation: Coral Disease Laboratory Analyses (Jan Landsberg, FWC FWRI) *Results in this study are ongoing, please contact Jan.Landsberg@MyFWC.com for updates.

- Jan provided a table of all samples that have been collected to date and of samples that have been processed by histology and transmission electron microscopy (TEM).
- Lesion samples of *Montastraea cavernosa* were identified histologically in the deeper sections of coral tissue.
- There is a large abundance of endolithic algae present in the diseased corals. The amount of endolithic algae in diseased corals could be quantified for comparison with healthy corals.
- Present work is examining the soft tissues, skeleton, and zooxanthellae to try to identify a pathogen.
- Preliminary histological data for *Montastraea cavernosa* shows the presence of lesions at the deeper basal body wall (BBW) including the gastrodermis were sometimes present in the absence of surface lesions. BBW lesions were also found in *Diploria labyrinthiformis*, *Siderastrea siderea*, and *Colpophyllia natans* (but only a small sample size was examined). The lesions have the putative appearance of liquefactive necrosis (as if digested by a lytic compound from e.g. a bacterial pathogen).
- No obvious bacteria were seen in histological sections using a variety of special stains, including Gram stain and routine hematoxylin and eosin stains, but bacteria were found in the surface mesoglea by TEM of one *Montastraea cavernosa*.
- Diagnostics are in the early stages and new disease identification procedures need to be developed.

Presentation: Coral Disease Treatment (Greta Aeby, University of Hawaii) *Results in this study are ongoing, please contact Greta@hawaii.edu for updates.

- Transmission study with Smithsonian on *Montastraea cavernosa* concluded that the disease is infectious via waterborne transmission and by contact.

- The pathogen is likely bacterial (histology ruled out larger-sized pathogens such as fungi's or ciliates): a study with Blake Ushijima (Oregon State) showed that antibiotic treatment stopped the progression of disease in all four experimental *Montastraea cavernosa* colonies, while disease continued to progress in control colonies.
- Of the 145 bacterial isolates that have been screened on corals so far, 3 have been shown to cause tissue loss on *Montastraea cavernosa* and *Orbicella faveolata*. Next steps are to identify protective microorganisms that may serve as a mechanism for differential pathogen-host susceptibility and to compare the microbial communities associated with healthy and diseased corals.
- Funding would be needed to expedite the continuation of Blake's work to fulfill Koch's postulates.

Presentation: Overview of Management Needs (Joanna Walczak, DEP):

- Acknowledging that there are many questions that need to be answered regarding this event, a reminder was given that the investigation also needs to address management needs. Given the limited funding and capacity, the discussion needs to focus on developing methods to answer the priority management questions such as:
    - What is (are) causing the disease(s)?
    - Are there multiple pathogens?
    - How is the disease spreading?
- Discussion focused on the issue that identifying the pathogen(s) could take years, so it shouldn't be communicated to leadership as a short-term management priority (however, pathogen identification efforts should continue simultaneously with other priority efforts).
- It was generally agreed that focusing on intervention strategies that could slow down or stop the event is currently the highest management priority.

Discussion: Sampling and Analysis Needs (Amber Whittle, FWC FWRI)

- Review of sampling methods [Ananda Ellis]
    - Sediment analysis around corals
        - Water quality from the sediment may help identify possible toxins and pollutants [Cheryl W.]
    - DNA swab for molecular and microbiome
        - DNA swabs may not work well underwater [Josh Voss]. Scraping the tissue and sucking it up for DNA may be a more effective alternative [Erinn M.]. The sampling method of scraping coral tissue and sucking it up can also be used for mucus samples and water samples if you filter out some of the seawater. However, the method works better for fleshy large polyps [Karen Neely].
    - Water collection near colony
    - Mucus and tissue samples
        - It is important to gather relevant environmental information that is useful when examining samples – just have the sampling without having habitat characteristics isn't as useful [Greta A.]
- Samples must be preserved quickly for DNA analysis.
- Macroalgae and symbiodinium samples are excluded from these sampling methods.
- The sampling methods listed above (sediment analysis, DNA swab, water collection, and mucus and tissue samples) were used in 2003 for an *Acropora* disease outbreak with no

luck. Further lab work needs to be the focus to identify a pathogen, then sampling can be more useful [Margaret Miller]

- Is it possible to compare *Meandrina meandrites* and *Dichocoenia stokesi* to *Montastraea cavernosa* to identify a pathogen [Rob Ruzicka]?

Break-out Groups: Sampling Plan Design

Attendees were separated into four groups and asked to devise a sampling plan for the disease outbreak.

Break-out Groups Report-Out on Sampling Plan Design

Group 1:

- This sampling plan incorporates management and learning by focusing effort on intervention to slow and stop the progress of disease spread while opportunistically obtaining disease tissue samples (via intervention techniques) to fulfill both needs. Extensive surveys are needed to locate the "brain, star, and elliptical corals," beyond the current disease boundary; since a large field effort would be needed, citizen science could be utilized to observe and monitor for these species. Once located, and visual signs of disease confirmed, management would act to treat or remove the diseased corals from the reef. Any diseased corals that are removed should be used in lab experiments to identify a pathogen. Action needs to be focused in the middle keys to stop the disease from spreading to the lower keys. The disease boundary should be continuously monitored for progression, as well as other sites that are not observed by 'citizen science' divers. While this management strategy is ongoing, sampling at other sites should continue.

Group 2:

- This sampling plan focuses on virulence rather than identifying a specific pathogen, and suggests processing samples that have already been collected rather than focusing on collecting new samples. To address site-specific questions, sites should be characterized as Pre-Irma and Post Irma, Pre-Disease and Post-Disease.

Group 3:

- This is a two-tiered sampling plan separated into two objectives: (1) identifying a pathogen, and (2) using water and sediment samples to understand the spread of disease.
- Sampling to identify a pathogen:
    - Collect live samples of the most affected coral species that have great enough densities.
        - Suggests that Mote Marine Lab to hold the live corals
    - Collect cores for histology, DNA, and microbiome analysis
    - Collect *Siderastrea siderea* to compare lesions with WP, confirm whether this is the same disease
    - Collect samples of apparently resistant species such as *Porites spp*. to investigate why they are resistant
    - Diseased corals at the southern boundary should be sampled to better understand the depth of the lesion and whether entire colonies are affected
    - Limited sites should be selected to reduce outside confounding factors, this sampling plans suggests limiting sampling to Tennessee Reef and Coffins Reef.

Group 4:

- The tissue samples that have already been collected need to be processed. Additional samples should be collected from around the boundary (in front of the boundary, on the boundary, and in areas where the boundary has already passed), with an emphasis on high sample quantity (collect as much data as possible, with backup samples to ensure quality), rather than on few samples from many sites. The species sampled will vary by site depending on abundance (n=5 is ideal); rare species should be collected regardless of abundance. This sample plan also suggests conducting full benthic cover surveys to complement sample data. Due to the large and unspecific scale of this sampling plan, management and resources (such as research cruises instead of daily dive excursions) are important considerations.

All plans assume that sampling will progress from 'clean' (apparently healthy) sites to 'dirty' (disease present) sites, and that all dive gear should be bleached between sites to avoid spreading the disease.

Discussion: Synthesize a Master Sampling Plan

- Site Selection: Sites should include the southern disease boundary, 2-3 sites where disease is present and 2-3 unaffected patch reefs (to target high coral diversity). Ideally, sites will be near CREMP sites but not within transects. Reference sites will be added in the lower Keys where the disease has not yet been observed.
- Sample Collection: Live coral colonies will be collected for transmission studies and will also provide samples for transcriptomics and microbiome analysis. N=5 of apparently healthy or diseased per site is ideal. Water samples will also be collected at each site, along with coral demographic surveys. Renting a live aboard or NOAA vessel may be most efficient.
- Target Species: (4 priority species in bold)
    - ***Dichocoenia stokesi***
    - ***Colpophyllia natans***
    - ***Montastraea cavernosa***
    - ***Orbicella faveolata***
    - *Diploria strigosa*
    - *Siderastrea siderea*
    - *Diploria labyrinthiformis*
    - *Orbicella annularis*
    - *Meandrina meandrites*
- Lab Analysis:
- Lab analyses decided on were histology, sequencing of microbiome, transcriptomics of *Montastraea cavernosa* and *Diploria clivosa*, TEM samples, Endolithic community, water microbiome, and genotyping samples.
- Specific people that could be used for the different sampling methods were suggested
    - Transmission studies in the lab- Valarie Paul, Erinn Muller, Cynthia Lewis, Nicole Fogarty, Joana Figueiredo, Abby Renegar, Josh Voss
    - Microbiome- Julie Meyer and Jose Lopez
    - Endolithic Algae- Kate Hubbard, Valerie Paul's Post Doc
    - Gene Expression Eukaryotic- Josh Voss
    - Gene Expression Bacterial- Julie Meyer
    - Histology- Jan Landsberg, Esther Peters
    - Field- FWC, Mote, Greta Aeby, Gilliam Lab, Valerie Paul

Priority Sampling Plan Workshop (Part 2)

November 8, 2017

Facilitator: Karen Bohnsack/Joanna Walczak

Attendees: Greta Aeby (University of Hawaii), Karen Bohnsack (DEP FCO), Ananda Ellis (FWC FWRI), Sarah Fangman (NOAA FKNMS Superintendent), Dave Gilliam (Nova Southeastern University), Lindsay Huebner (FWC FWRI), Kristi Kerrigan (DEP CRCP), Yasu Kiryu (FWC FWRI), Vladimir Kosmynin (DEP DWRM), Jan Landsberg (FWC FWRI), Lauri MacLaughlin (NOAA ONMS SEGOM), Erinn Muller (Mote Marine Lab), Karen Neely (Florida Keys Community College), Francisco Pagan (DEP CRCP), Valerie Paul (Smithsonian Institution), Rob Ruzicka (FWC FWRI), Stephanie Schopmeyer (FWC FWRI), Jennifer Stein (The Nature Conservancy), Joshua Voss (Florida Atlantic University), Joanna Walczak (DEP FCO), Brian Walker (Nova Southeastern University), Amber Whittle (FWC FWRI).

Welcome

Amber Whittle recapped the discussion from day two and reviewed the draft sampling master plan.

Presentation: Southern Boundary Identification Monitoring (Karen Neely, FKCC) *Results in this study are ongoing, please contact Karen.Neely@fkcc.edu for updates.

- FKCC has been tracking disease progression based on *Dendrogyra cylindrus* data.
- The disease outbreak began in 2014; by 2016 the disease was spreading at a rate of 30km/year. If the disease continues to progress at this rate, Sombrero will be affected by July 2018.
- As of August 2016, the disease was spreading at a rate of 4.5km/month. At the time, Tennessee Reef was the edge of the outbreak.
- After Irma, Tennessee Reef was infected by disease.
- Next week FKCC will conduct parch reef sampling for disease on every coral above 4 cm on a 10m belt transect, while a roving diver will scan for the priority species listed at this workshop.

Presentation: Cheeca Rocks Photo Mosaics (Karen Bohnsack, DEP FCO) *Results in this study are ongoing, please contact Karen.Bohnsack@dep.state.fl.us for updates.

- Photo mosaics of Cheeca Rocks were produced by stitching together a collection of high-resolution images of the reef. Though the images take a while to process and stitch, the end result is a highly detailed mosaic of the entire reef with resolution down to single coral heads. Previously, NOAA collected five years of photo data to capture multiple bleaching events. Now that Cheeca has begun to show signs of disease, the technology will be utilized to capture the outbreak. Unfortunately, the first set of disease-focused images were taken after Irma, so the disease may already be present in the first data set.

Presentation: Disease and Fate tracking in Martin County (Josh Voss, FAU) *Results in this study are ongoing, please contact jvoss2@fau.edu for updates.

- This study used fate tracking and transcriptomics to examine gene expression during sub-lethal stress (freshwater exposure) in *M. cavernosa*.
- *M. cavernosa* was the focus due to the transcriptome being already established.

- Date was the biggest change in gene expression during the study. This correlated with high rain amount, which means the corals grow poorly and cannot repair well during low salinity fluctuations.
- Corals at St. Lucie were beginning to become diseased in 2017, post Irma most corals apart of the fate tracking were affected or killed.

Presentation: Coral Monitoring (Valerie Paul and Greta Aeby) *Results in this study are ongoing, please contact Paul@si.edu for updates.

- Tagged and monitored individual corals with different disease patterns and found that Z-spar was a good method for creating a fixed location on the coral to measure the effects of disease/ lesion progression.
- *Montastraea cavernosa* has a lot of variation in disease morphology that changes overtime.

Discussion: Fixed Site Data Collection

- Site Selection:
  - It is important to identify fixed sites available for repeated sampling and/or monitoring. Ideally, sites would be paired with long-term monitoring sites so that findings can be bolstered by pre-existing long-term data. If possible, 3 replicate sites should be selected within each habitat type to provide statistically defensible data.
  - Two types of sites: (1) monitoring/fate tracking (without manipulation) at CREMP/SECREMP sites (targeting patch reefs in the Keys due to high diversity and large coral colonies in southeast Florida) and (2) sites for collection/potential intervention at the leading and lagging edge of the disease front.
- Sombrero Reef was identified as a site for experimental treatments. Studies should be implemented in the near future to figure out what may be effective against the disease, so that we can take a "last stand' at Sombrero Reef. If disease jumps past Sombrero, it is likely to continue spreading through the lower Keys. Data Collection:
  - Three types of data collection efforts were discussed: Spatial epidemiology mapping "boxes" to model transmission dynamics, repeated measures transect for demographic data collection, and individual colony fate tracking for species specific information, documenting rates of tissue loss, etc.
  - Tagging and detailed monitoring for disease transmission can be done within the pre-established CREMP/SECREMP transect; however, any manipulation would have to be done to corals outside the transect (sites will be split: mapping within the transects, sampling outside the transects).
  - Spatial epidemiology maps should be created for the sites. This map will identify every coral within a given area of the site and colonies selected for intervention should be well beyond the generally mapped area so as not to influence the community dynamics of the site being mapped. The size of the mapping boxes should be the maximum size feasible, based on how many corals are at a site; generally, this should be between 10 and 25 m^2.
- Target species:
  - *Meandrina meandrites* and *Dichocoenia stokesi*
  - In southeast Florida, *Orbicella faveolata* and *Pseudodiploria strigosa* (common large colony species).
- Frequency of Data Collection:
  - Sites should be monitored weekly for spatial epidemiology mapping and individual colony fate tracking both before they are infected and during the initial disease outbreak; monitoring can be reduced to monthly after the disease event has moved through the

most susceptible species and has started impacting *Montastraea cavernosa*, *Siderastrea siderea*, etc.

Closing Remarks:

- The goal is to keep Florida's corals alive, collaborate, and share results to try to find answers to the outbreak.
- Obviously, the efforts discussed over the last few days are more than we currently have funds or capacity for, but all of the recommendations will be compiled into a comprehensive Coral Disease Response and Management Plan for the Florida Reef Tract.
- The next step will be to prioritize the actions, and move forward on funding as many of the recommendations as possible; slowly chipping away at the list of priorities.
- Partners were encouraged to seek out funding and openly collaborate for these priority projects to ensure that the groups capacity is leveraged as much as possible.