Florida Reef Tract Coral Disease Outbreak

Coordination Meeting #4
November 3, 2016
12:00 – 2:00pm

Meeting Summary

Attendees: Cliff McCreedy, Anna Toline, Meaghan Johnson, Tracy Ziegler, Lonny Anderson, Bill Goodwin, Lauri MacLaughlin, George Sedberry, Margaret Miller, Dana Wusinich-Mendez, Caroline Rogers, Meghan Balling, Kristi Kerrigan, Francisco Pagan, Melissa Sathe, Mollie Sinnott, Daron Willison, Ana Zangroniz, Karen Bohnsack, Joanna Walczak, Janice Duquesnel, Trudy Ferraro, Vladimir Kosmynin, Jeff Beal, Vanessa Brinkhaus, Yasu Kiryu, Jan Landsberg, Erin McDevitt, Kerry Maxwell, Tom Reinert, Ken Banks, Kathy Fitzpatrick, Rebecca Ross, Sara Thanner, Dave Gilliam, Brian Walker, Josh Voss, Danielle Dodge, Karen Neely, Esther Peters, Carrie O'Neil, Cory Walter, Lad Akins, Dan Clark, Stephanie Clark, Ed Tichenor, Jennifer Stein, Wes Brooks, Jane Fawcett, Lindsey Precht

Welcome, Roll Call, Meeting Purpose

- Karen Bohnsack welcomed everyone to the call and noted that the purpose of the coral disease coordination calls is to improve information sharing and response coordination for the ongoing coral disease outbreak in Florida. The disease has been present in southeast Florida, specifically in the vicinity of Miami-Dade County, for over 2 years. While this has continued to evolve during that time – including spreading to new locations, impacting different species, and varying in prevalence, the rate of progression, and the lesion appearance, there are sites in Miami where this event was first observed that are still being affected over 2 years later.
- The agenda for the call will include an update on disease observations, updates on response efforts (including coral tissue sampling and coordination with the USGS National Wildlife Health Center), as well as updates on the NSF Rapid Grant Proposal, Data Aggregation effort, and the preliminary results from the analysis of coral tissue samples collected in 2015 which will be presented via a live webinar. As time allows, there will also be an opportunity to discuss other reef issues, and to discuss the interpretation of recent versus old mortality.

Update on Florida Reef Tract Disease Observations

- Southeast Florida – *Kristi Kerrigan (DEP CRCP*)
    - Kristi Kerrigan noted few reports were received over the past month due to bad weather. The reports that were received were mostly associated with the Florida Reef Resilience Program (FRRP) and Reef Visual Census (RVC) monitoring programs.
        - One report of *Montastraea cavernosa* (MCAV) with white plague was received in Palm Beach County.
        - A total of seven disease reports were received from Broward County. Species included MCAV, *Colpophyllia natans* (CNAT), *Porites* spp., *Acropora palmata* (APAL), *Dendrogyra cylindrus* (DCYL), and *Dichocoenia stokesii* (DSTO). Additionally, a *Pseudodiploria strigosa* (PSTR) was observed with tissue loss but no active disease margin. Other reports noted observations of dark spots, or no active disease but recent mortality.

        - Three reports were received from Miami-Dade County, which included observations of MCAV and *Solenastrea bournoni* (SBOU) with white plague.
    - Kristi noted that although there were few reports during this period, disease has generally been observed spreading north and south, and also extending onto the 3rd reef at ~80' depth.
    - Reporters are encouraged to submit SEAFAN reports of sites with NO disease in addition to reports with disease.
- Biscayne National Park – *Karen Bohnsack (DEP) on behalf of Vanessa McDonough (BNP).*
    - The bad weather has largely hindered the National Park Staff's ability to get out on the water; of the few days they were in the water there were no changes to previously reported conditions or new emergence of disease observed.
- Florida Keys – *Cory Walter (Mote Marine Laboratory)*
    - Cory Walter noted that while there was not much new information due to weather issues, two reports were received from the Key Largo Elbow and Key Largo Dry Rocks.
        - At Key Largo Dry Rocks, diseased *Acropora cervicornis* (ACER) and *Siderastrea siderea* (SSID) with the "white blotch" disease were observed.
        - CNAT and SSID with white disease were also reported at Key Largo Elbow. Although the number or percentage of diseased colonies was not reported, the observer did note that up to 50% were showing signs of thermal stress. It is unknown if that was actually bleaching or disease; this needs to be verified.
        - There were no signs of disease at a couple of FRRP sites off of Key West.
    - Karen Bohnsack reported that the information from Key Largo Dry Rocks was corroborated by Coral Restoration Foundation staff who were at that site near the end of October. During an hour of snorkeling, no SSIDs bigger than 20 cm were observed unaffected by disease.
    - Additional information was received from staff at the Florida Keys National Marine Sanctuary that active white disease was still present on SSIDs around Carysfort toward the end of October.
    - On behalf of Derek Manzello, Karen Bohnsack also reported that as of the end of September, Cheeca Rocks still looked unaffected by disease.
- Dry Tortugas – *Karen Bohnsack (DEP) on behalf of Mike Feeley (NPS)*
    - There is no update from the Dry Tortugas over the past month, largely due to weather. The National Park Staff are filling in the details from their September disease monitoring trip and should be able to share that information during the next call.
- Karen Bohnsack reminded those making disease observations that photos to accompany reports of disease observations are important. For sites that are routinely visited, time-series photos of the same colonies with a ruler for measuring the rate of tissue loss would be very helpful. Reports of sites that are unaffected are also important.
- Questions/Comments:
    - Jennifer Stein (TNC) mentioned that the FRRP Disturbance Response Monitoring (DRM) data should be available soon. The summer 2016 Quick Look Report, which will have additional disease information, will also hopefully be out by next week. The report will also be available on www.frrp.org.

- Karen Bohnsack remarked that the Quick Look Report would also be distributed to the group via email along with the other the follow-up information from this meeting.

Update on Current Response Efforts

- Coral Tissue Sampling – *Vanessa Brinkhuis (FWC)*
  - FWC found a couple thousand dollars to cover travel and boat costs for 3 FWC employees to do a small scale disease sample collection to compliment what they did in July 2016 and the samples that were sent to FWC in 2015.
  - Three full field collection days are planned to target: 1) A diseased site in SEFL (likely offshore Broward County) to collect some actively diseased white plague samples; 2) A healthy reference site at the northern boundary of the disease outbreak (if they are unable to find a disease-free site in this range they will settle for a site with normal background disease levels, and target apparently healthy colonies from that site); and 3) Healthy reference samples from outside the current disease outbreak area (likely a healthy patch reef off the Middle Keys). If weather is an issue, the Middle Keys site may get pushed to spring 2017.
  - For disease samples, they will target MCAV, SSID, DLAB and CNAT colonies since they will be collecting on the FKNMS permit in the Middle Keys and are restricted to those four species which were also targeted during the July 2016 Upper Keys disease sampling.
  - In southeast Florida they will also be collecting a few healthy samples from MMEA and EFAS if they can be found, and a few other species of apparently healthy samples as comparative reference samples for samples that were collected in 2015 and sent to FWRI.
  - Questions/Comments:
    - Vanessa emphasized that FWC is trying to find disease-free reference sites at the northern and southern boundaries of the outbreak area, and requested that if anyone observes an unaffected site in northern Broward County with the targeted species (SSID, MCAV, CNAT, DLAB) to please share that information.
    - A Middle Keys site has already been identified for the southern reference site.
- Coordination with USGS National Wildlife Health Center – *Joanna Walczak (FDEP)*
  - Joanna Walczak reported that a formal request was submitted on behalf of the state for epidemiological and sample analysis support from the NWHC. They have acknowledged receipt of the request and we anticipate a call in the next week or two to discuss what resources and capacity they can provide.
- By way of other updates on response activities previously discussed, Karen Bohnsack noted that Cindy Lewis sent a summary report about the pillar coral (*Dendrogyra cylindrus*) rescue effort, which will be shared with the follow-up email to this coordination call. There are no other new updates since the last call; disease is still occasionally appearing on some of the fragments. The status of the wild colonies is currently unknown since the weather has hindered field activities.

Working Group Updates

- NSF RAPID Grant Proposal – *Karen Bohnsack (DEP) on behalf of Valerie Paul (Smithsonian Institute)*
    - Karen Bohnsack reminded attendees that during the last call it was reported that the NSF Biological Oceanography Program declined our project concept for RAPID funding to support additional sampling and analysis.
    - Since then, the NSF Working Group has reconfigured the project and submitted a summary of the project plan to the Symbiosis, Defense, and Self Recognition program in the National Science Foundation, which has expressed interest in the project and has invited a full proposal to be submitted. The proposal will investigate host-pathogen interactions and will include field, water quality, molecular and histology components.
    - The goal is to submit the proposal by Thanksgiving, and if successfully funded would be initiated this winter.
- Data Aggregation –
    - Karen Bohnsack reminded attendees about the previously discussed challenge of having a variety of different data sets and the need to aggregate that data to more accurately reflect the status of the disease outbreak. Kristi Kerrigan has taken the lead on this effort.
    - Update and TAC Meeting Outcomes – *Kristi Kerrigan (DEP CRCP)*
        - Kristi Kerrigan provided an update on her progress with this issue, noting that she has begun investigating methodologies, protocols, and parameters collected by the different monitoring programs. Work on this effort will continue.
        - This issue was also recently brought to the attention of the Southeast Florida Coral Reef Initiative's (SEFCRI) Technical Advisory Committee (TAC). Esther Peter's presented her Comprehensive Conditions Report, which included an overview of the variety of datasets that exist in southeast Florida before and during the disease outbreak, and the TAC discussed next steps with this data in the context of the disease outbreak.
        - In particular, the TAC looked at a list of management questions to determine if we have the appropriate data to answer those questions, identified missing information, and discussed whether the disease outbreak is one event or 2 separate events (given the progression of the disease and new manifestations observed, the current outbreak may be unrelated to the previous event).
        - Members of the TAC noted that what is currently being observed may not be white plague, but something entirely different such as yellow band disease, and agreed that it is important to determine if bacteria is a primary pathogen or a secondary invader via electron microscopy and histology.
        - The TAC also discussed limitations in the FRRP methodology to effectively capture disease, including a possibility that it does not accurately capture coral density, surveys only occur once per year, and that there are no recurring stationary sites. The TAC recommended that post-bleaching surveys be conducted in winter 2017 (even though bleaching in summer 2016 was not severe) to capture disease impacts. While the TAC noted that FRRP data can

answer about half of the disease-related management questions, some modifications to the methodology would allow more questions to be answered.

- Kristi Kerrigan also mentioned that John Fauth's PhD student, Danny Gooding, is doing his dissertation on the FRRP data set, which includes a spatial analysis and an investigation of annual changes to visualize hotspots in disease and bleaching across the reef tract.
- Questions/Comments:
    - Joanna Walczak highlighted a key point that when dealing with these complex events, one data set cannot give us all the answers. Multiple types of data are needed to understand these big events, so Joanna cautioned against drawing conclusions from one individual data set. None of the existing data sets were designed to capture this type of event, so in some cases we're trying to force the data to do something they were not designed to do.
    - Dan Clark inquired as to whether sedimentation and proximity to different projects has been analyzed as a contributing factor.
        - Karen Bohnsack noted that Esther Peter's Current Conditions Report is identifying a variety of data sets that exist during the 3 year period when the disease outbreak occurred. While it is not currently being analyzed, if those data exist they will be part of a larger analysis. Joanna Walczak added that with support from the NWHC, we hope someone can look at the bigger picture to see where connections might exist and identify areas for more focused analysis.

- Disease Prevalence Mapping – *Karen Bohnsack (DEP)*
    - Karen Bohnsack provided a summary of a separate project going on that is relevant to the data aggregation issue: a Florida Reef Tract-wide resilience assessment that is looking at existing data to determine the relative resilience of our reefs based on various indicators.
    - One of the indicators being looked at is disease prevalence, and in summarizing disease information for that project, similar problems arose in that there are lots of different data sources, collected with different methods at different times. In order to best capture disease prevalence, the principal investigator on the project has agreed to first incorporate data sets that are standard across the whole reef tract (e.g., from FRRP, CREMP/SECREMP stations, and NCREMP) to draft a map that is color-coded based on average total disease prevalence (using a scale of very low, low, medium, high, and very high). In order to capture some of the more localized data collection efforts, we will also be reaching out to our partners who have been collecting data to provide input as to what their data or observations suggest the average disease prevalence is in different regions.
    - Through this combination of quantitative data and expert judgement a final map will be generated that will help us understand the spatial variation in peak coral disease prevalence along the Florida Reef Tract. This information will then also be tied into a communications piece which will include the maps and

publicly digestible information about the disease outbreak, management efforts, etc.

- Sample Analysis Working Group: Preliminary Findings From 2015 Samples (Skype Webinar) – *Jan Landsberg (FWC)*
    - Jan Landsberg presented a map of locations where the various samples that were received in 2015 were taken, in order to provide some context to what is observed from the histological sections. Although some of the samples did not work out, sampled species included MCAV (2), MMEA (2), EFAS, PSTR, and PAST.
    - These are very preliminary results.
    - Sample 1: MCAV (#3)
        - Jan noted that various comparative stains are used to show different histological features of the corals and to potentially identify organisms of interest within the coral tissue, including mucocytes (mucus-producing cells). In particular the different stains used included H&E, Giemsa, Thionin, Alcian blue, and modified Gimenez.
            - H&E slide (routine slide): Mucocytes typically appear white and open.
            - Giemsa and Thionin: The mucus appears dark, possibly because it targets the organisms within the mucocytes. In these stains the putative elementary bodies (coccoid shape) are visible and appear to "stream" while the putative reticulate forms appear to be present inside the mucocytes. There are other photos from the cnidoglandular band where the reticulate bodies appear to be outside the mucocytes.
        - Images of these organisms are presented at different magnifications throughout the presentation. Similar organisms seem to be present in four of the species examined (and rare in the fifth), although it is unknown if it is the same organism, or closely-related organisms.
        - At higher magnification what appears to be stages of "rickettsia-like organisms" (RLOs) are observed, although this is speculative until electron microscopy and molecular identification can be completed. It is unknown if what is being observed are multiple organisms or stages of the same organisms.
        - Rickettsia-like organisms are obligate intracellular bacteria, which have 2 stages in their life cycle. It is unknown if this is what is being observed on the slides, but there appear to be two morphological types: elementary bodies (infective, transmission stage; once they infect the cell they become intracellular) and reticulate bodies (intracellular stage within the coral mucocyte). These can readily multiply. While the elementary bodies appear small, uniform in size, and organized, the reticulate bodies are bigger and appear darker-stained.
        - The organisms that appear on the slide could also be other intracellular bacteria that are separate species from the putative rickettsia-like organism.
        - These organisms are absent from certain sections of the coral; they seem to target specific tissue and cell types.
    - Sample 2: MCAV (#1)
        - There was a lot of degenerate tissue and necrosis, and residual mucus. There was not much tissue left, although within the areas that appeared to be dead or dying there were still areas of apparently heavy infection. Where the stains

show up as black or streaks (overstained), this was interpreted as being the elementary or reticulate bodies. The mucus may have been infected where it is very heavily stained. Electron microscopy will help with this interpretation.
        - There may be other co-associated organisms, such as fungus in the skeleton, opportunistic ciliates, and other biota.
        - At the simplest level perhaps there is one primary pathogen affecting multiple coral species, but this may not be the case.
    - Sample 3: MMEA (#4)
        - This specimen appeared heavily affected, especially in the epidermal areas (versus gastrodermis). This is not quantifiable, but rather subjective; there is an obvious visual difference.
        - Similar reticulate-looking bodies (appear like grapes) and smaller elementary type bodies are apparent in this specimen.
    - Sample 4: MMEA (#4)
        - Heavily affected areas were observed in the cnidoglandular band.
        - The magnified view shows what appears to be affected mucocytes.
    - Sample 5: EFAS (#5)
        - Streaks of elementary bodies are possibly being observed. Electron microscopy will be necessary to clarify what these are.
    - Sample 6: PSTR
        - Less affected, except in cnidoglandular band, but with lots of mucus and thick surface mucus layer.
    - Sample 7: PAST
        - Least affected. A lot of necrosis and masses of mucus. Occasional rickettsia-like organisms were observed, but these may be normal symbionts or background infection.
    - Overall, these observations are just the beginning of looking at what organisms may be present in the coral tissues and what their potential role is in the current disease outbreak. A lot of work remains to quantify, identify, and determine if these organisms are pathogenic.
    - Esther Peters has previously described a rickettsia-like organism in the acroporids. These appear similar to what was observed in these samples, but it is unknown if they are the same species or not.
    - Questions/Comments:
        - In response to an inquiry from Vladimir Kosmynin about how the 2015 tissue samples were taken, Karen Bohnsack clarified that no comparative healthy tissue samples where obtained, only samples that appeared diseased. Jan Landsberg and Vanessa Brinkhuis noted that the 2016 sampling effort targeted a suite of samples, including both a histology and molecular sample from the disease margin, and a histology and molecular sample from an unaffected area. These additional samples will help with context in understanding what appears on the histology slides, including which groups of microorganisms are present in diseased tissue and their functional role (e.g., can they kill cells, do they proliferate in response to certain conditions, etc.?). There currently appear to be indications of heavily infected versus unaffected areas.

        - Esther Peters noted that she has some old sections of MCAV that were apparently healthy. While some of the suspect microorganisms may be present at times in apparently healthy tissue, a great increase in mucocytes and associated microbes are observed in the 2015 slides. It is still unknown if these are the disease agents. Microbes can have different effects on coral tissue, which still needs to be studied further. Additional analysis of these slides is necessary to figure out what is going on.
        - Esther Peters reminded attendees that molecular analysis of the microbes will be necessary to determine what is in the tissue. There may be different kinds of microbes and their roles are currently unknown. It is important to consider that an infection of the mucocytes means that the coral cannot produce as much mucus as normal, which is important for controlling interactions with microbes. The infected mucocytes may not be able to produce the quality and quantity of mucus that is necessary to keep other pathogenic organisms at bay. There is still a lot to be learned on this subject. It is evident in these slides that there is a severe infection of the coral mucocytes, which affects the coral's ability to stay healthy and remain alive.
    - The 2016 samples are still in process but should be ready for review soon.

Other Reef Issues

- *Xestospongia muta* Disease/Algal Blooms:
    - Kristi Kerrigan noted that SEAFAN received two additional reports of *Lyngbya* blooms in Palm Beach and Broward counties. Sponge disease was also reported.
- Interpreting Recent vs. Old Mortality:
    - Karen Bohnsack introduced this issue that was brought up via an email conversation; recent versus old mortality has implications for what can be attributed to the current disease outbreak versus what may have been dead long before.
    - Vladimir Kosmynin clarified that this is important to address this issue because there is a big audience with different experience collecting field observations. Some mortality that has been attributed to disease is actually sedimentation death (e.g., Slide #6 in the Photo PDF). Photographs with scale bars are important when attempting to identify disease. There are many other stressors in south Florida and partial mortality is common.
    - Questions/Comments:
        - Esther Peters reiterated that it is important to document as much information as possible in the field. Photo documentation of changes overtime is especially important, but difficult to do.

Wrap-Up and Adjourn

- Karen Bohnsack provided reminders and reviewed action items from the call:
    - A special webinar presentation on coral disease and management will be presented via webinar by Greta Aeby on November 14th from 3:00-4:30 PM EST.
    - Karen will send final minutes from Disease Coordination Meeting #3, and draft minutes from this call (Disease Coordination Meeting #4), and the Pillar Coral Rescue Summary Report.

- Karen will send a calendar invite for the next coordination call (still TBD)
- Reminder: Everyone who spends time in the water, should continue to submit reports to SEAFAN and C-OCEAN. Observations of disease, bleaching, algal blooms, etc. can all be submitted, as well as information about sites NOT affected by disease.