Florida Reef Tract Coral Disease Outbreak

Coordination Meeting #5 January 18, 2017 1:00 – 3:00pm

Meeting Summary

Attendees: Anna Toline, Amanda Bourque, Vanessa McDonough, Jeff Miller, Tracy Ziegler, Derek Manzello, Billy Causey, Lauri MacLaughlin, George Sedberry, Kurtis Gregg, Jocelyn Karazsia, Margaret Miller, Tom Moore, Alison Moulding, Dana Wusinich-Mendez, Cheryl Woodley, Ilsa Kuffner, Lauren Toth, Caroline Rogers, Valerie Paul, Kristi Kerrigan, Kelly Montenero, Francisco Pagan, Daron Willison, Karen Bohnsack, Joanna Walczak, Ivana Kenny Carmola, Vladimir Kosmynin, Janice Duquesnel, Trudy Ferraro, Jeff Beal, Lisa Gregg, Lindsay Huebner, Yasu Kiryu, Jan Landsberg, Cindy Lewis, Erin McDevitt, Brian Reckenbeil, Bill Sharp, Tom Reinert, Kathy Fitzpatrick, Dave Gilliam, Brian Walker, Karen Neely, Esther Peters, Ana Zangroniz, Ed Tichenor, Caitlin Lustic, Jennifer Stein, Lindsey Precht

Welcome, Roll Call, Meeting Purpose

- Karen Bohnsack welcomed everyone to the call and reminded attendees 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. First reported in 2014, this continues to be an issue in early 2017.
- The agenda for the call included reports on recent disease observations, updates on response efforts (including coral tissue sampling, coordination with the USGS National Wildlife Health Center, and the results from the 2016 Florida Reef Resilience Program Disturbance Response Monitoring [FRRP DRM]), working group updates (including the status of the NSF RAPID Grant Proposal and initial findings from coral tissue sample analysis), and a summary of other ongoing reef issues.

Update on Florida Reef Tract Disease Observations

- Southeast Florida Kristi Kerrigan (DEP CRCP)
 - Kristi Kerrigan noted that SEAFAN received reports at seven sites since the last call.
 - Palm Beach County One report was received in November, which observed Montastraea cavernosa (MCAV), Siderastrea siderea (SSID), and Pseudodiploria strigosa (PSTR) with white plague-like disease. Only one coral colony was found without signs of disease, and there were several colonies with 100% recent mortality. Depth ranged from 55 – 70'.
 - Broward County A total of five reports were received, four of which were from sites that had been repeatedly surveyed every few months. Observations included SSID with white plague and dark spots, as well as white plague affecting PSTR, MCAV, Orbicella faveolata (OFAV), and Porites astreoides (PAST). Orbicella faveolata (OFAV) was also observed with 100% mortality. At one site, over 80% of MCAV and OFAV colonies were dead or dying, and a large colony of Dendrogyra cylindrus (DCYL) was observed with >85% mortality from white

plague. An additional report from Lauderdale-by-the-Sea noted four colonies of MCAV with white plague.

- Miami-Dade County One report was received off Miami Beach where an unspecified mounding coral was observed with white plague.
- Biscayne National Park Vanessa McDonough/Amanda Baroque (BNP)
 - Disease continues to be widespread in Biscayne National park, with all depths and hard bottom habitat apparently affected (continuous and patch reefs). In December, BNP staff revisited 7 of the 8 sites with known colonies of DCYL. All were 100% dead. Plans are underway to visit the remaining site.
 - At the deeper sites (60 80') visited over the past month, few colonies of the massive species are still alive. The condition of MCAV is especially dire; most are dying. There seem to be two different types of disease: in addition to the clear band of disease progressing across the colonies, there is a condition where the polyps appear to be "melting" in which they shrink back into themselves and die. Most of the colonies at depth are affected by the later condition.
 - At the shallower sites where SSID is the most common remaining massive coral species, most of those colonies are also diseased and dying.
 - Karen Bohnsack noted that a photo of the "melting" MCAV condition will be sent with the follow-up information following the call (photo courtesy of Amanda Baroque).
- Florida Keys Karen Bohnsack (DEP) on behalf of Cory Walter (Mote Marine Laboratory)
 - Karen Bohnsack summarized the disease reports that from the Keys since the last call.
 - Upper Keys A number of reports were received from the Upper Keys, including observations from November, December, and early January. Affected sites included Mosquito Banks and Higdon's Reef in John Pennekamp Coral Reef State Park, Molasses Reef, Pickles Reef and Davis Reef. Species reported with disease included SSID, PSTR, PCLI, DSTO, DLAB, DCYL, and MCAV, among others. Reports were primarily of white disease, but dark spots and recent mortality were also observed. There were varying levels of severity, but generally reports ranged from 50% of these species affected to most or all of the observed colonies. At Molasses, *Acropora palmata* appeared unaffected. Images of the disease progression at Davis Reef were included with the Disease Photos PDF sent to all participants (photo credit: Brian Reckenbiel).
 - Middle Keys Disease affecting SSID and other species has been reported as far south as Sombrero Reef. Two reports were received in early December. Only one diseased SSID was observed at Sombrero Reef, while CNAT was observed with disease at a patch reef off Grassy Key. More reports are needed between Tavernier and Marathon.
 - Lower Keys In early January, Mote Marine Laboratory staff revisited a site west of Looe Key where they previously observed white disease on 10+ OFAV, and a few Colpophyllia natans (CNAT), PSTR, and PCLI back in July. During this repeat visit, no active disease or recent mortality was observed. No noticeable signs of disease were observed during site visits at several patch reefs in the Lower Keys.
- Dry Tortugas Karen Bohnsack (DEP) on behalf of Mike Feeley (NPS)
 - National Park Service Staff are still working on an updated report from their last monitoring event in Dry Tortugas National Park; that information will be shared as soon as it's available.

- Ilsa Kuffner (USGS) noted that during a visit to Pulaski Shoal in mid-December, there was no evidence of disease in shallow habitat (12 – 20'). The status of sites previously identified with disease was unknown.
- Questions/Comments:
 - Valerie Paul (Smithsonian Institute [SI]) inquired as to whether anyone has seen any indication that the disease is slowing down with the cooler weather.
 - Amanda Baroque (BNP) responded that there does not seem to be any sign of improvement.
 - Karen Bohnsack (DEP) requested additional input on that question this during the next call.
 - Valerie Paul (SI) agreed this would be good to know. Some diseases show seasonality while others do not.
 - Vladimir Kosmynin (DEP) inquired if anyone has observed *Siderastrea radians* (SRAD) affected in the same way as the SSID?
 - Lauri MacLaughin (ONMS) reported no observations of this in the Upper Keys or other sites visited this year.
 - Lindsay Huebner (FWC) noted observations of some SRAD in the Upper Keys with pale patches and some red spotting, but it did not look as intensely dark as the condition affecting SSID.
 - Ester Peters (GMU) noted observations of SRAD with dark spots, which appears redder on SRAD than SSID. The tissue loss that is occurring on the SSID has not been observed.
 - Karen Bohnsack (DEP) requested additional input on this during the next call. A
 photo of impacted SRAD will be sent following the call (photo courtesy of
 Lindsay Huebner).

Update on Current Response Efforts

- Coral Tissue Sampling Karen Bohnsack (DEP) on behalf of Vanessa Brinkhuis (FWC)
 - Karen Bohnsack reminded attendees that during the last meeting it was reported that FWC found a small pot of money for additional sampling. Plans were to target a diseased site in southeast Florida, and healthy reference sites in both southeast Florida and the Middle Keys.
 - FWC staff were able to get out in Broward County in November for this additional sampling. They chose to sample a diseased site near, but outside of, the Southeast Florida Coral Reef Evaluation and Monitoring Project (SECREMP) site BC4. There was no problem finding diseased colonies, but difficulty in finding healthy colonies. White plague and bleaching band disease were present, and many 100% recently dead colonies were observed. Given time constraints, prevalence surveys were not conducted; focus was placed on sample collection.
 - Colonies affected with white plague and unknown bleaching band disease were targeted for sampling. In the end, they collected from 3 healthy and 3 diseased colonies of SSID and OFAV; and from 5 healthy and 10 diseased (i.e., 5 white plague, 5 bleaching band) colonies of MCAV.
 - They collected tissue cores for histology, molecular, transmission electron microscopy (TEM), and transcriptomics, and mucus samples from MCAVs and SSIDs. All molecular

samples were shipped to Val Paul/Francois Seneca at Smithsonian Research Station for microbial analysis and transcriptomics. Mucus samples were divided and half sent to Valerie Paul; the others remain in the -80°C freezer at the Florida Fish and Wildlife Research Institute (FWRI). Histology and TEM samples are at FWRI. Histology samples have been decalcified but still need to be sectioned and embedded to produce slides.

- As far as future work:
 - FWC will plan one additional day to obtain reference samples in the Middle or Lower Keys (at the southern boundary of the disease outbreak area), which will coincide with their surveys of the Coral Reef Evaluation and Monitoring Project (CREMP) long-term monitoring stations in late spring.
 - A disease-free reference site at the northern outbreak boundary has not been identified and effort will not be placed on sampling here unless someone can provide numbers to a healthy reef north of Broward (although local feedback indicates that Broward is completely affected). Sampling near Palm Beach would be limited to nearshore areas to avoid logistical complications of sampling in current.
 - More funding will need to be identified for additional sampling efforts (e.g., Palm Beach reference sampling, any additional disease sampling).
- Coordination with USGS National Wildlife Health Center Joanna Walczak (DEP)
 - During the last call it was noted that a request had been submitted to the USGS National Wildlife Health Center (NWHC) for assistance with this disease event. Since then, DEP and partners have engaged in productive discussions with the NWHC, which is based out of Madison, Wisconsin with a field office in Hawaii.
 - DEP briefed the NWHC staff on the Florida disease outbreak to date, who were surprised at the large scale of this issue. Given the huge scale and number of potentially contributory factors, we are trying to narrow the focus on some key sites with a lot of data (from CREMP/FRRP, etc.). NWHC staff have started looking through the data to help us understand what they would normally look for.
 - Despite the large amount of data available along the FRT, we've discovered that the data we have are not giving us the full picture of what's happening with this event. The NWHC is helping scope potential tweaks to existing monitoring protocols or determine if a new protocol is needed to improve disease surveillance. They will also be internally raising awareness in USGS for additional funding and support, and have offered to help with the histological analysis.
 - In addition to this immediate, short-term assistance, as a long-term project the NWHC has also offered to analyze the other data sets (e.g., temperature, water quality, etc.) available during this time period in an attempt to better understand patterns and identify potential causes. Such information can help inform what can be done better in the future to avoid a similar outbreak. They've initially identified the high turf algae cover on our reefs as a likely contributor. While short turf is less of a problem, longer turf traps sediment and causes herbivores to avoid those patches. This turf and sediment can harbor disease. We will be working with the NWHC to access all the relevant data layers, which may require assistance from others who house that information.

- Unfortunately, we do not have a good handle on what can be done to stop the spread of disease. There are potentially site-specific, intense, in situ responses (e.g., amputation) that could potentially help stop the spread into new sites, but it is unknown if this would be feasible or effective in the field.
- Questions/Comments:
 - Valerie Paul (SI) reminded attendees about the Smithsonian Institute aquarium white-plague outbreak that corresponded to the beginning of this outbreak along the Florida Reef Tract (FRT). Although it is easier to experiment with interventions in the aquarium system, amputation did not help. This does not mean it might not help elsewhere.
 - Regarding amputation, Esther Peters (GMU) noted that part of the issue is that we do not know what is impacting corals. Corals have gastrovascular canals that connect all of the polyps. On MCAVs with a bleaching margin you can see that the polyps adjacent to that lesion were becoming contracted (as though there were starting to become affected by something), while those further away were still extended. It is likely that something is spreading through the colonies. This is harder to see in this reaction in the SSIDs and other species. Studying the histology samples might help us learn more about what is going on.
 - Joanna Walczak (DEP) noted that when communicating about this disease outbreak, a common question is "what can we do about it?" Even in the absence of perfect information and definitive answers, at some point we might need to try some of the dramatic in situ techniques to see what might help. It is hard to allocate more money when we still have no viable means of stopping the spread. A pilot field intervention effort may be necessary to garner further funding and support for this response effort.
 - Valerie Paul (SI) reminded attendees about information shared previously about Cindy Lewis' lab-based interventions. She may help guide what does and does not work, and what may be translated to the field.
 - Karen Bohnsack (DEP) offered to reach out to Cindy Lewis and Cheryl Woodley to provide additional insight on this issue during a future call.
 - Cheryl Woodley noted that all DCYL in their lab are still alive.
 - Valerie Paul (SI) also noted that in addition to turf algae, the FRT has a lot of macroalgae and cyanobacteria that are directly toxic to corals.
 - Regarding algae, Lindsay Precht inquired if mats of algae have still been observed since the hurricane or cold fronts that have come through? They have not been seeing as much as previously off Miami Beach.
 - Kristi Kerrigan (DEP CRCP) noted that no new SEAFAN reports have been received on this issue.
 - In response to an inquiry, Karen Bohnsack (DEP) reminded attendees about reports of dense algae mats reported across portions of the reef tract during previous calls (including Miami, the Upper Keys, and Key West).

- Vladimir Kosmynin (DEP) inquired as to the species of this algae.
 - In Palm Beach County, it was tentatively identified as *Cladophora spp*. It is unknown if the observations were the same throughout the reef tract.
 - Vladimir Kosmynin clarified that *Cladophora spp.* is dense, long, and hair like, which is different from the red cyano mat present on page #9 in the Disease Photos PDF, and the short "toothbrush" like turf algae. Photos and more information on specifically where algae was observed (e.g., inner reef, etc.) would be helpful. This would be very unusual on the outer reefs beyond Hawk's Channel.
- Karen Bohnsack (DEP) noted that additional input on this issue will be requested during the next call.
- FRRP DRM 2016 Results Jennifer Stein (TNC)
 - Jennifer Stein noted that the Quick Look report for this year's Florida Reef Resilience Program Disturbance Response Monitoring (FRRP DRM) is available on the FRRP website under the publications tab.
 - A total of 162 surveys were completed across 28 zones. It was a mild to moderate bleaching year. Twelve zones showed moderate bleaching, and only two zones had severe bleaching (inshore reef and forereef in the Upper Keys). The prevalence of paling and bleaching decreased in all subregions compared to the past two years, except for Martin County (where prevalence was higher than 2015, but lower than 2014).
 - Disease prevalence in 2016 was the highest in the Upper Keys, where 15% of all surveyed corals had disease. In 2014, DRM showed that the highest concentration of disease was also in the Upper Keys (~5% of all surveyed corals). All subregions increased in disease prevalence in 2016, except for Broward-Miami and the Middle Keys.
 - There has been press generated from the Quick Look Report, including the Key West Citizen, Keys News, and the Associated Press. The Washington Post is slated to come down next week to dive with The Nature Conservancy for video footage and a story.
 - Questions/Comments:
 - In response to Jennifer Stein's inquiry about site recommendations to take the Washington Post reporter, Joanna Walczak (DEP) noted that targeting outreach to the high-profile dive sites (e.g., Molasses) might raise the alert status of this issue to the public, which may help garner further capacity and support.

Working Group Updates

- NSF RAPID Grant Proposal Valerie Paul (Smithsonian Institute)
 - Valerie Paul noted that the National Science Foundation (NSF) proposal was submitted last week, and clarified that the RAPID mechanism is intended for research concepts that need immediate support, versus those that go through the regular, lengthy NSF grants process.
 - The ultimate goal of the proposed project is to isolate and identify the causative organism for this outbreak, using methods that have been used successfully elsewhere.

- If funded, experimental aquarium work would occur at the Smithsonian Marine Station in Fort Pierce. The team includes Valerie Paul, Greta Aeby (University of Hawaii), Greta's former post-doc and his advisor, and Julie Myer (University of Florida).
 - Valerie Paul also noted that Julie Myer is already working on microbiome samples from coral tissue samples previously collected by FWC this summer. She has some sequencing funding from the Estee Lauder Award that she is applying to this project already. She can provide updates on this call once data are available.
- The timeframe for the project is to-be-determined, although the target start date is April 1st.
- Sample Analysis Working Group: Preliminary findings from 2016 Samples (Skype Webinar) Jan Landsberg (FWC)
 - Jan Landsberg noted that this analysis is from samples obtained from FWC's July 2016 collection at Grecian Rocks off Key Largo, where a series of diseased coral species, as well as some healthy colonies, were sampled for a variety of analyses (including histopathology, ultrastructural, and molecular work). This update includes preliminary information on histology from species not sampled in 2015, and new transmission electron microscopy (TEM) results.
 - SSID #19: Siderastrea siderea, July 22, 2016 Diseased Sample
 - Close-up macroscopic images of samples of unaffected and affected portions of the colony show that the diseased portions appear to collect more sediment material, although further review is necessary to look for a consistent pattern.
 - The microscopy and tentative organisms are similar to those observed in the 2015 samples. Using additional stains for better identification, chlamydialike/rickettsia-like organisms (CLOs/RLOs) appear to be present in the epidermis, cnidoglandular band, and mucocytes. There could be a lot of potential organisms; it is unknown if they are the same across species and samples. There is a lot of work still to be done to obtain parallel information from the histology and ultrastructure.
 - Initial TEM results show the same putative RLOs or CLOs at low magnification. Other organisms look similar or bacterial-like, but what is being observed may be very different. There is a lot of material that needs to be worked through to gain familiarity with normal-looking versus diseased tissue. This initial analysis shows the tentatively identified "elementary bodies" mixed with larger stages of the CLOs. Molecular work will help understand if the same organisms are being observed in the different coral species.
 - In RLO and CLOs, elementary bodies are smaller than the reticulate bodies and have an apparent nucleoid area (observable as a dark oval electron dense "nucleus" in the center of the body). A higher magnification TEM image (slide 8) shows a possible mixture of elementary and reticulate bodies. The elementary body is the infective stage in the water or cells; these have a growth stage to a reticulate body (the larger stage, which have more ribosomes in the periplasm – observable as dots in the bigger cells [presumptive reticulate bodies]; slide 9) which then divides to form new elementary bodies. This cycle repeats itself and eventually the elementary bodies are released into the water to infect a new

area or cell. This may be what is being observed in the light microscopy and ultrastructurally.

- Slide 10 shows a possible division stage (2 nucleoids in the same body), which may show the reticulate bodies dividing to form new elementary bodies.
- SSID #19: Siderastrea siderea, July 22, 2016 Unaffected Sample (Slide 11)
 - The same organisms are being observed in the unaffected areas of a diseased colony (e.g., the apparently healthy tissue): Presumed RLO/CLOs are present in the epidermal and cnidoglandular band areas of the corals.
 - TEM (slide 12) shows where the potential elementary bodies seem to "channel" next to the mucocyte. This is similar to the "streaming" discussed with the initial 2015 sample analysis. Additional input is necessary to understand how best to describe this anatomically.
 - Light microcopy observations need to be compared to the ultrastructure; analysis of more sections from each specimen, comparison to normal tissue, and consultation with ultrastructure experts will help with understanding what is being observed.
 - In November, all samples collected for light microscopy have parallel samples for electron microscopy and ultrastructure. There are a multitude of specimens that could be shared with others who can assist with analysis. Analysis of these samples will give a better picture of what is being observed in the light microscopy.
 - We do not know what relationship these presumed RLO/CLOs have with the disease outbreak, but they are present in most of the tissues that have been analyzed. This is not necessarily normal.
- o MCAV #12: Montastraea cavernosa, July 21, 2016 (Slide 17)
 - Macroscopic photos show the disease vs. unaffected samples. *Halofoliculina* were observed on the diseased sample (this is common on post-disease corals).
 - In the unaffected areas, reticulate and elementary bodies still seem to appear in the tissue. A new stain, Macchiavello is specific for CLOs. Stains will need to be matched across the same sections to ensure that no granular cells or other host cells are being misinterpreted with these organisms.
 - In this sample and in the MCAV sample from 2015, unidentified organisms were observed; while they might be reticulate bodies, they are larger and they do not look to be associated with the surface where the RLO/CLO's have been observed. While they were prolific in some specimens, they were not seen in every MCAV sample, nor have they been seen on any other species. It is unknown if these are a stages in the CLO lifecycle or separate organisms.
 - Fungal infections in the skeleton were also common in the 2015 and 2016 samples. Esther Peters (GMU) and Thierry Work (USGS) will start working through the slides.
- CNAT #15: Colpophyllia natans, July 22, 2016 (Slide 23)
 - There were no CNAT samples collected in 2015.
 - Histologically, similar issues are being observed with CNAT as with other species, including CLOs in the epidermis and gastrodermis, fungal infection, and a heavy mucus layer.

- o Summary: Last slide
 - CLO/RLO (chlamydia-like/rickettsia-like organisms) appear to be present in diseased and unaffected areas of the same colonies in multiple coral species. Not quantified, subjective observation only. Samples from healthy specimens not yet examined.
 - CLOs/RLOs need molecular identification
 - Are located more commonly in cnidoglandular band epithelium and in the epidermis
 - Additional unidentified organisms (stages?) also observed in some MCAV. Relationship (if any) to CLO/RLOs or fungus unknown. Note other pathologies, parasites, pathogens not reported.
 - Fungal infections commonly present in the skeleton of multiple coral species. Not quantified, subjective observation only.
 - These are preliminary data and interpretations.
- o Questions/Comments:
 - Lisa Gregg (FWC) inquired whether Esther Peters is involved in these analyses.
 - Jan Landsberg: Yes.
 - Esther Peters mentioned previous work on *Acropora* spp. and noted that similar RLOs are now being found in these other species. There is a paper by Forest Rohwer's lab that showed RLOs found in some other species (Esther will look back at which species). She has observed them in cnidoglandular bands over the years but did not know what they were. Now special stains, fluorescent in-situ hybridization with probes for different bacteria groups (EB-2 probe for RLO/CLO), etc., are helping with understanding. The fact that unaffected areas of the coral have these same RLOs could be an indication of an early phase of infection. Unaffected and affected areas will need to be compared. Rickettsia and Chlamydia are pathogenic across multiple species.
 - Lisa Gregg reiterated that corals may be affected even if they are not showing outward signs. This is important to consider when moving specimens around; we may unknowingly be transmitting disease.
 - Esther Peters noted the importance of including histologic sampling and microbiology in addition to visual protocols. Funding and capacity make this difficult to accomplish.
 - Jan Landsberg also highlighted the risk of transmission in the field if this is a RLO/CLO.
 - Ilsa Kuffner noted a recent meeting with the USGS Dive Program Lead meeting regarding decontamination protocols. They are working on identifying alternatives to bleach. We need protocols for scientists moving among diseased sites.
 - Karen Bohnsack reiterated the importance of following decontamination protocols, and noted that updates on non-bleach decontaminants will be reported during the next call.

- Xestospongia muta disease observations:
 - Vanessa McDonough (BNP) noted that into December they were still seeing ~10% of Xestos affected by disease, ranging from early infection to complete crumbling. They will continue to monitor for this during additional fieldwork.
 - Kelly Montenero (DEP CRCP) explained that the picture in the Disease Photos PDF is from December in Broward County; no new reports have come into SEAFAN in 2017.
- FKNMS Backcountry Sponge Kill:
 - Bill Sharpe (FWC) noted that they have been doing sponge restoration research in Florida Bay. In mid-December, they observed a sponge die-off in the vicinity of Sandfly Key (located at the west end of 7-mile Bridge and a few miles north). Two genera were affected, including *Hercenia spp*. (vase and brown branching) and *Spongia spp*. (which includes the commercial species: glove, yellow and sheepswool). Necrotic tissue was observed and mortality of these genera was 100%. A 5 square-mile area was surveyed to understand the range of the die off; disease prevalence ranged from 30% to 100%. No impacts were observed in the Middle Keys area of Florida Bay. Sponge cuttings were moved from the unaffected Marathon nurseries into the diseased area, and are being tracked on a weekly basis. One site showed impact, but subsequent monitoring showed that whatever caused the die-off may have abated (that partially impacted has not shown additional impact in the last few weeks). Tissue samples have been sent to FWRI.

Wrap-Up and Adjourn

- Karen Bohnsack (DEP) provided reminders and reviewed action items from the call:
 - Karen will send a follow-up email that will include notes from today's call (Disease Coordination Meeting #5), the final meeting summary from the last call (Disease Coordination Meeting #4), a link to the FRRP DRM 2016 Quick Look Report, and photos of the MCAV with the different-looking disease and SRAD colony with potential disease.
 - Request recommendations for diseased sites for TNC's field excursion with the Washington Post, and healthy reference sites along the northern boundary of the disease outbreak.
 - Reports of disease should be submitted to SEAFAN and C-OCEAN.
 - A calendar invite will be sent for the next call.