Florida Reef Tract Coral Disease Outbreak

Coordination Meeting #6
March 1, 2017
11:00 AM – 1:00 PM

Meeting Summary

Attendees: Amanda Bourque, Vanessa McDonough, Meaghan Johnson, Derek Manzello, Lonny Anderson, Lauri MacLaughlin, Billy Causey, Kurtis Gregg, Margaret Miller, Jennifer Moore, Dana Wusinich-Mendez, Cheryl Woodley, LeAnn White, Thierry Work, Meghan Balling, Kristi Kerrigan, Francisco Pagan, Daron Willison, Aubree Zenone, Karen Bohnsack, Joanna Walczak, Trudy Ferraro, Vladimir Kosmynin, Jeff Beal, Vanessa Brinkhaus, Yasu Kiryu, Jan Landsberg, Erin McDevitt, Michael Bollinger, Brian Reckenbeil, Kathy Fitzpatrick, Rebecca Ross, Sara Thanner, Jena McNeal, Dave Gilliam, Brian Walker, Mauricio Rodriguez-Lanetty, Cindy Lewis, Karen Neely, Esther Peters, Julie Meyer, Ana Zangroniz, Saundra Sample, Ed Tichenor, Caitlin Lustic, Jennifer Stein, Valerie Paul, Mike Dixon, Bill Precht

Welcome, Roll Call, Meeting Purpose

- Karen Bohnsack welcomed attendees to the call and reviewed the agenda and attachments sent prior to the meeting.
- The agenda includes reports on recent disease observations, discussion regarding outstanding questions from Disease Coordination Call #5, updates on response efforts (including coordination with the USGS National Wildlife Health Center, FWC's disease outbreak investigation, pillar coral rescue, and Coral Disease Health Consortium [CDHC] disease interventions), and initial molecular sample analysis findings.
- Attachments include: Meeting agenda, PDF of recent photos, updated Miami disease surveillance information, draft coral disease decontamination protocol from USGS, and a summary of the pillar coral status and disease.

Update on Florida Reef Tract Disease Observations

- Southeast Florida – *Kristi Kerrigan (DEP CRCP*)
    - Kristi Kerrigan noted that generally the report from southeast Florida is similar to the previous call; it is difficult to tell if the disease is slowing down since less people have been on the water. Observations of no disease have been reported at deeper sites in Broward and Palm Beach counties.
- Biscayne National Park – *Vanessa McDonough/Amanda Bourque (BNP)*
    - Biscayne National Park staff reported that over the last month the disease has not appeared to be getting worse; no new diseases have emerged and the previously affected corals are dead or dying so the disease is less apparent.
    - Although not particularly common, *Mycetophelia* spp. appear to be doing well. For the most part they seem to be vibrant and healthy while other species in the same area are struggling. Anecdotally, the disease conditions have abated.
- Florida Keys –
    - John Pennekamp Coral Reef State Park – *Trudy Ferraro (DEP FPS)*

- Trudy Ferraro explained that John Pennekamp Coral Reef State Park has 6 major reef sections and that park staff have conducted surveys at two sites within each section. During these 20-minute surveys, species present are noted, as well as the percentage of those species affected by disease or bleaching. A total of 24 species were present across the 12 survey sites; 5 species had no observable disease (including *Agarcia lamarcki* [ALAM], *Myctophyllia* spp., *Orbicella franksi* [OFRA], *Porites divaricata* [PDIV], and *Siderastrea radians* [SRAD]). Of the 12 sites, these unaffected species were only present at one or two locations. Disease was observed at all sites, with Turtle rocks and Higgins Reef being the most affected
- Pennekamp staff plan to revisit these 12 sites between May and June.

o Florida Keys National Marine Sanctuary – *Karen Bohnsack (FDEP) on behalf of Cory Walter (Mote Marine Laboratory)/FKNMS Staff*

- Karen Bohnsack summarized reports received within FKNMS since the last disease call.
- Upper Keys:
  - Disease reports continue in the upper Keys, including Davis, Molasses and Pickles reefs. Affected species are similar to what has been reported previously, including *Orbicella* spp., *Montastrea cavernosa* (MCAV), and essentially all of the brain corals [*Diploria labyrinthiformis* (DLAB], *Meandrina meandrites* [MMEA] and *Pseudodiploria* spp). Most to all observed colonies of *Siderastrea siderea* (SSID) and MMEA were diseased or dead.
  - Since the last call, disease was also reported affecting the Aquarius deep reef (at Conch Reef) between 70 and 90 feet; many species showed disease although the *Acropora cervicornis* (ACER) looked good.
- Middle Keys:
  - White disease was recently found on 4 of the 16 genets of ACER at FWRI's Long Key nursery near Tennessee Light, each of which was previously documented to be highly susceptible to disease transmission. No disease was visible at this site 3 weeks prior. Diseased tissues were removed and the site will continue to be monitored.
  - Relevant to this report, Diego Lirman (RSMAS) noted that they first saw a similar rapidly advancing white disease in November at their Key Biscayne nursery; it has now abated, but they did lose 15% of their corals. Samples are preserved in Z-fix.
- Lower Keys:
  - Mote Marine Lab staff visited 20 patch reefs around the lower Keys during February, including inshore, mid-channel and offshore. Disease still seemed largely absent, although most of the SSID showed some sort of stress, including dark spots or white spots that looked like paling or damselfish bites. Some brain corals (*Colpophyllia natans* [CNAT] and *Pseudiploria strigosa* [PSTR]) appeared paling or bleached with some mortality on the ridges, but it was hard to determine if there was recent mortality on some of them. Photos of these conditions were included in

the photos PDF and input requested from the other attendees on these observations.

- Pillar Coral (*Dendrogyra Cylindrus* [DCYL]) Population Status – *Karen Neely, FKCC*
  - Karen Neely reported that two weeks ago, she and staff from Florida Aquarium visited every known DCYL site from Biscayne National Park through the 7 Mile Bridge (~55 sites). Lystina Kabay (NSU) also checked the status of known DCYL sites north of BNP; there is only one small colony still alive within BNP and north. South of BNP, through Turtle Rocks, Carysfort, Elbow Reef, French Reef, and Molasses everything was mostly dead with some disease still observed. At Conch Reef, however, there was a change in the predominant condition from mostly dead, to very active disease with recent mortality.
  - There is no DCYL between Conch Reef and Crocker Reef (10 miles south of Conch), but at Crocker Reef there was a noticeable change in both DCYL and other species. At this location, the DCYL looked good and healthy *Dichocoenia* stokesi (DSTO) and brain corals were also observed, although some background disease was still present. This information provides an indication as to the current southern boundary of the disease outbreak area.
  - Karen Neely highlighted a document sent out to the group with graphs and photos, which shows the status of all sites with known DCYL over time, including 2014-2015, August 2016 and February 2017. This shows the progression in disease severity within each site, and helps illustrate the spread of disease across all known DCYL sites along the Florida Reef Tract (where the northern-most sites have been the most severely impacted, while the southern-most sites are relatively unchanged). This documents also contains anecdotal information regarding other species observed at these sites, which shows the same pattern of disease and mortality in the upper Keys, with improved status from Crocker Reef south. This document also shows the total DCYL losses across the Reef Tract since 2014, including 99% of colonies (91% of genotypes) in the southeast Florida region, 75% of colonies (59% of genotypes) in the upper Keys, and 20% of colonies (13% of genotypes) in the middle Keys. A number of genotypes are still alive in the genetic bank but extinct in the wild.
  - Three different forms of disease seem to be apparent on the DCYL: classic white plague with a clearly defined tissue margin, rapid tissue loss sloughing off in sheets, and yellow blotchy series of lesions with a less defined disease margin. It is unknown if these are observations of the same or different diseases; input is requested from other attendees on this matter.
  - Questions/Comments:
    - LeAnn White (NWHC): Have you collected tissue samples from these different DCYL lesions for histology (to determine if the disease is different)?
      - Karen Neely: Yes, as part of the DCYL rescue project tissue was brought in from a lot of different colonies, especially those with the rapid tissue loss. Many of these samples lot are being held at Cheryl Woodley's lab in South Carolina.

- Dry Tortugas – *TBD*

- Meaghan Johnson (DTNP) noted that no disease has been reported at Pulaski shoal or the coral nursery. No updates were available on the status of diseased sites previously identified in the Dry Tortugas.

Follow-Up to Call #5 Inquiries:

- Karen Bohnsack reminded attendees that there were a couple of outstanding inquiries raised during the January call, including: requests for input or other reports of the "melting" MCAV condition in BNP, information on observations of diseased SRAD, and whether there were any signs of the disease slowing down.
- Great Star Coral (*Montastrea cavernosa*) "Melting" Condition
  - The general consensus was that this is a dead colony of MCAV that has been overgrown by a Clionid sponge, as indicated by the oscula in the photo. It is relatively common that Clionid sponges will not affect the morphology of the coral calices. A similar condition has been observed affecting CNAT, where the sponge preserved the morphology of the skeleton fairly quickly. Data show that these sponges increase after coral mortality events and that they prefer recently dead coral for settlement substrate compared to old dead coral.
- Lesser Starlet Coral (*Siderastrea radians*) Disease Observations
  - No other reports have emerged of paling or spotting affecting SRAD. Attendees were asked to keep an eye on this species and report any updates.
- Indications of Slowing Disease Progression?
  - Reports indicate that disease continues to remain active in the upper Keys, as new reports and sites have continued to emerge during the cooler winter months.
  - Observations from the 60-80' reefs in Biscayne National Park report that disease conditions seem to have abated, and/or affected colonies are now dead so (qualitatively) disease is less apparent.
  - FWRI staff observed disease at Sombrero in December, but as of February, those corals that had active disease were no longer losing tissue and had no active disease visible.

Update on Current Response Efforts

- Coordination with USGS National Wildlife Health Center – *Joanna Walczak/Karen Bohnsack (FDEP)*
  - Joanna Walczak provided an update on the ongoing coordination with the USGS NWHC. NWHC staff have begun analyzing long-term data sets. We previously reported that initial analyses of the 2014 and 2015 data did not show a signal; we are waiting on the 2016 and eventually the 2017 data to see if that will change the story. Indications are that 2016 SECREMP data will show a signal. They are also developing a simulation model to see if any tweaks need to be made to the existing long term data collection protocols, or what would need to be in place to improve disease surveillance across the Reef Tract in the future.
    - LeAnn White (NWHC) clarified that with Florida's low coral cover it is difficult to detect a change in disease prevalence in the data. They have written the code for a preliminary simulation model which is currently being reviewed internally. While most support thus far has been done in-house as staff are available, the NWHC is planning to apply for an NSF Graduate Research Fellow position to

assist with a larger spatial analysis of available data. Proposals are due March 17th and will be shared with agency staff prior to submittal.

- Regarding the general question of "what can we do about this?" that is important for decision-makers, Karen Bohnsack summarized recent guidance from the NWHC on how to more effectively communicate about the ongoing coral disease outbreak in Florida, it's transmission, and what can be done about it:
  - Generally, we do not know what is killing the corals as this is a very complicated field of study. Regarding transmission, we have no credible data that this disease is caused by an infectious agent; it may be an environmental problem or a combination of factors. Data from sample analysis will help inform what is contributing to this disease.
  - As far as interventions, a long-term goal is to continue to fund research to determine the causes of coral disease, although it will likely be years before we may have answers. Still, this is a critical component as with any disease, key pieces of information need to be figured out to make informed management decisions to reduce the burden of that disease moving forward. We have not yet reached this point with coral disease; we are in the 1700s as far as the state of knowledge about coral diseases.
    - As an analogy: It took 20 years to arrive at the cause of AIDS. In the first 10 years alone, knowledge about what was contributing to the condition was entirely wrong (e.g., high stress, lifestyle choices, etc.). Credible interventions were only developed many years later once it was determined to be a virus.
    - Similarly, until the mid-nineteenth century, most scientists thought that harmful swamp gases caused malaria - the word means 'bad air' in Italian. Eventually, scientists figured out that malaria is associated with a parasite in the blood cells and that mosquitos are the vector. Understanding these key pieces of information was necessary before the disease could be better managed in people via interventions such as mosquito control, bed nets, etc.
  - It is misleading to say that any in-situ intervention would solve the current coral disease outbreak; this is not realistic. The most realistic option for moving forward is to continue to work on the issues that we can control that we know are impacting corals. There is a lot of documented evidence about the factors that affect coral survival so the best thing we can do is to reduce these stressors to make the conditions on the reef more conducive to coral recovery and survival. This is analogous to clean drinking water, which is responsible for the biggest advance in human health in history. This mentality stops at the coastline.
- Questions/Comments
  - Brian Walker (NSU) noted that trying to change water quality will take decades, and inquired as to whether there are any plans for in-situ interventions.
    - Karen Bohnsack clarified that this is the big picture goal; there is a lot of other work that is being done or that potentially could be done in the field in the meantime. This information is important for framing the

issue with decision makers to justify further research and recognize that coral reefs are connected to a broader system.

- Billy Causey (NOAA ONMS) noted that during a previous outbreak of black band disease a Looe Key between 1986-1989, Harold Hudson came up with the aspirator to remove the disease, which was then followed with modeling clay to block the disease margin. Most of these efforts were successful removals, but it was very labor intensive. Still, there are some in-situ mechanisms that can address disease, although white band and yellow band disease do not have a clear target for removal, so it is more complicated. Mote Marine Lab, Esther Peters and others also tried to look at the microbial complex to see if it was possible to change the microbial community from negative to positive during these types of events. There are things that can be done, it just takes innovative thinking; in-water interventions should not be discouraged. Meanwhile, the sources of this disease are very complex and need to be seriously investigated.
    - Karen Bohnsack agreed that this is an important component and encouraged attendees to share new ideas during upcoming coordination calls.
- Margaret Miller (NOAA SEFSC) mentioned that even in the absence of perfect knowledge about the origin or etiology of white diseases, we do know that some corallivores appear to transmit these diseases. Thus, predator control is an appropriate in situ control mechanism to consider. This type of intervention may not have a broad impact, but it is important to keep in mind that vector control is an option that exists in the absence of understanding where these diseases are coming from.
    - Billy Causey supported this idea.
- Esther Peters (GMU) highlighted that while there are some in-situ things we can do, it is important to still look at the bigger picture. The Keys have changed a lot in the last century with urbanization. Messaging this change, the impact of urbanization on these habitats, and what can be done to reduce those impacts is also important.

- Joanna Walczak provided some additional information from recent meetings with USGS staff during the U.S. Coral Reef Task Force meeting in Washington D.C. USGS has offered to lead us through a decision support process, which will provide guidance in how to look at all the environmental issues at play and help us understand how to focus our limited capacity to better respond to this disease. We are considering offering an overview of this process via a webinar during the next call; then a smaller group of managers may be invited to participate in the actual process. This may help identify next steps forward in coordinating a response.

- FWC Coral Disease Outbreak Investigation –*Vanessa Brinkhuis (FWC)*
    - Vanessa Brinkhuis highlighted a collaborative effort with Mauricio Rodriguez-Lanetty's (FIU) lab at to conduct additional sampling at Conch Reef and analysis. FWRI staff provided Mauricio's group with the appropriate protocols and equipment for field collection and preservation (histological and molecular samples). A time-series set of molecular samples from specific colonies at Conch Reef is available, which will provide a good opportunity for comparison with diseased samples. Sampling of *Orbicella*

faveolata (OFAV) and *Agaricia* agaricites (AAGR) has been added to the exiting FKNMS permit, in addition to the previously permitted SSID, MCAV, DLAB, and CNAT.
    - Mauricio Rodriguez-Lanetty reported that they visited Conch Reef last week to conduct surveys and understand the magnitude of the disease outbreak. The work is ongoing and those results will be shared when available. The collections are targeted for next week.
    - Karen Bohnsack reiterated the interest in collecting samples at Conch Reef due to the availability of historical, healthy samples for reference, and requested information from the group regarding other archived samples from before the disease outbreak that may be preserved for histology or molecular analysis. It will be useful to be aware of these types of samples, should there be a need to use them as a reference point for interpreting the current diseased samples.
  - Vanessa Brinkhuis also noted that FDEP has identified additional funding to support FWRI's disease investigation efforts. The goals of the project are:
    - 1) Development of standardized protocols for coral tissue sample collections for histopathology, ultrastructural, and molecular analyses.
    - 2) Collection of reference samples from healthy corals at a disease-free site in southeast Florida and from a disease-free site south of the disease outbreak area in the middle Florida Keys.
    - 3) Tissue processing - Histological slide preparation of collected samples, including preparing and shipping two sets of standard histology slides for collaborators (Thierry Work and Dr. Esther Peters) and a subset of special slides for fluorescent in-situ hybridization (Dr. Esther Peters).
    - 4) Continuation of this investigation to provide insight on the disease(s) and potential pathogens from gross field observations using detailed morphological descriptors, and from histopathology and ultrastructure (transmission electron microscopy [TEM]) for microscopic and pathologic diagnosis in the laboratory.
    - 5) A summary of preliminary results shared in a final report by June 30th; these preliminary results and protocols will also be distributed to the wider group.
  - There is still a need to find a disease-free reference site in SEFL – please keep any eye out.
- Pillar Coral (*Dendrogyra cylindrus*) Rescue Update – *Karen Neely (FKCC)/Cindy Lewis (KML)*
  - Cindy Lewis (KML) provided an update on the DCYL rescue effort, including ex-situ quarantines at Keys Marine Lab. During the week long field-survey and collection effort by Karen Neely and the Florida Aquarium staff, 74 fragments (from isolated pillars) were brought in from 19 sites across the upper and middle Keys. These sites were categorized and the associated fragments distributed as follows:
    - Category 1 Sites = No disease observed on the site, the DCYL colonies, or the fragments that were obtained. The highest priority was to immediately get these fragments into an in-situ nursery – two fragments were hung immediately at the Coral Restoration Foundation (CRF) nursery. Total # of Category 1 fragments = 15 (6 sites). CRF and FWC each have 2 fragments, Florida Aquarium has 8, and 3 are in Charleston, S.C. to preserve those genotypes, as multiple fragments were obtained from the same colonies.

        - Category 2 Sites = Disease was observed elsewhere on the site or on the DCYL colonies, but the collected fragment appeared healthy. These were transferred to the ex-situ nursery for quarantine. These will hopefully eventually be transferred to an offshore nursery. Total # of Category 1 fragments = 22 (5 sites). Florida Aquarium has 21 of these, and 1 is in Charleston, S.C.
        - Category 3 Sites = Diseased present on the site, the DCYL colonies, and the collected fragments. Total # of Category 3 fragments = 37 (8 sites). Thirty-four of these fragments are in Charleston, S.C.
    - Some genotypes have been completely lost to this disease event, in the wild and in captivity. The current living genetic bank, which includes the spring/summer 2016 collections includes 203 primary fragments (from 44 sites). Twelve of these are identified genotypes, 27 are still unknown, and some were unable to be sampled before they died. All samples will be genotyped to try and maintain the genetic diversity.
    - Karen Neely clarified that in 2014 there were an estimated 142 genotypes on the reef. Currently 51 of those are in the genetic bank, but it is estimated that greater than 50 genotypes have been lost for good. More work needs to be done to bring additional genotypes from the lower Keys into the bank. This is planned for the next few weeks.
- Disease Intervention Updates – *Cheryl Woodley (NOAA)*
    - Karen Bohnsack reminded attendees about a previous update from Cheryl Woodley and her team during the September call.
    - Cheryl Woodley provided an update on the status and success of on-going lab-based interventions. They have received sick corals from three separate collection events, including:
        - 1) Fragments from BNP – 5 fragments received from the same genotype. Three of these are doing well; this is the only known remaining living tissue of that genotype.
        - 2) Fragments from the July 2016 DCYL rescue effort – With this effort, there was an attempt to improve the treatment approach. Currently, 22 fragments remain from 11 different colonies. Just less than half of the representatives were lost from this collection.
        - 3) Fragments from early 2017 DCYL rescue – A total of 38 fragments from 11 different sites were received from the third rescue effort (three Category 1, one Category 2, and the remainder were Category 3). To date, only 5 of the really sick fragments have been lost.
    - New, more aggressive treatment protocols have been implemented, including surgery to remove disease margins on site as soon as the fragments were brought in. This was followed by an antiseptic Lugol's dip and transfer to artificial seawater before transport to Charleston.
    - A series of experiments were conducted using different antibiotics. Paromomycin had mixed results previously and was not effective during the most recent treatment. A second experiment involved different concentrations of Ampicillin dissolved into 20 separate tanks, although everything was moved within 6 days. The third treatment included a dental paste-type formulation that was used as a vehicle to apply Amoxicillin. This has been successful since it can be applied directly to the tissue margin versus

dissolved in the tank water and changed daily. Although there is still a lot to learn, overall success has improved since the first rescue.

- Questions/Comments:
  - Relative to the previous conversation about field interventions, Margaret Miller (NOAA SEFSC) inquired about the potential to try the dental paste-type application in the field.
    - Cheryl noted that this particular paste works best if applied with a syringe when the coral fragment is dry or damp (it is activated and gels upon hitting water). There has been some effort to figure out a formulation that can be applied underwater. If anyone wants to try the existing formulation, Cheryl will send them some. There is a shelf life for this product, however, so the application would have to happen soon.
  - Margaret Miller stressed that this approach may be worth pursuing and noted interest in participating in a field trial if such an effort was permitted.
    - Cheryl: Without knowing what is causing this disease, we're shooting in the dark with what type of compound to incorporate. We are using a drug, but it may not be the right drug to combat this disease; there may be other effective chemicals that could be incorporated. The current drug does have a time-release ability, which is helpful.
  - Billy Causey inquired as to the use of Tetracycline for this application (it has been successfully used in fish).
    - Cheryl Woodley was unsure if this would be effective, as some drugs are a problem for the zooxanthellae. There are a series of drugs that have not been effective with the DCYL treatments.
    - Val Paul (SI) noted that they did experiment with adding Tetracycline to the water, but the corals did not respond well.
    - Cheryl Woodley noted that some treatments are worse than not treating the corals.
  - Margaret inquired if it would be possible to put together a plan to test this type of approach via a field trial, noting that treatment could be tested on DCYL and some other affected species.
    - Cheryl inquired as to the status of the molecular analysis, noting that Jan Landsberg (FWC) had hypothesized the presence of a chlamydia-like organism in the histology. This may indicate a different pathogen, which is helpful in planning treatments and interventions.
    - Thierry Work (USGS NWHC) emphasized that from 982 coral tissue samples from the Indo-Pacific, there was no evidence that bacteria were involved with tissue loss. This is important to keep in mind when discussing antibiotic treatments; this may not be tackling the root cause of the disease.
    - Cheryl Woodley agreed that we cannot say that bacteria are the primary cause; it may be secondary. However, corals have survived when treated, and have died when not treated.

- Val Paul inquired if Greta Aeby may have had success identifying bacteria that have contributed to tissue loss.
- Thierry reiterated that there is no real evidence in histology that the bacteria is killing corals. There is a disconnect between what pathologists would expect to see on bacterial diseases in animals, and what the coral ecology community thinks is killing corals.
- Esther Peters(GMU) reiterated the importance of considering the primary versus secondary pathogen(s), as well as the biotic versus abiotic disease agents. Hopefully electron microscopy from FWRI will show if there are viruses involved.
- Jan Landsberg remarked that it may be premature to conduct field tests since we cannot know the repercussions on other organisms in the food chain, and because there may be multiple pathogens that require different treatments. These questions should first guide experimental work in the lab.
- Chery Woodley noted that Paromomycin was tried because it has a fungal and bacterial aspect.

▪ Margaret Miller agreed with the security concerns with applying antibiotics in the field, but cautioned attendees to remember the rate at which corals are dying. There is a need to consider balancing a small environmental risk with the real risk that some species are facing local extinction. This is a discussion that needs to happen, the group should consider what the risks are of applying a field trial versus the risk of not doing anything.

- Vanessa Brinkhuis (FWC) suggested starting with some experimentation in a more controlled nursery-type environment.
- Cindy Lewis also cautioned about the potential creation of antibiotic-resistant microbes and suggested first testing these applications in flow-through tanks. There will likely be push back to adding antibiotics to the water.
- Cheryl Woodley noted that there are non-antibiotic antimicrobial agents that are being tested on corals (possibly even in a field setting). There are other kinds of compounds that may be effective that we have not considered.

o Karen Bohnsack summarized the need for more discussion on this topic and volunteered to connect the smaller group of interested parties after the call to discuss offline. Any new treatment successes from Cheryl Woodley's group or elsewhere will continue to be of interest to the group.

- Decontamination Protocols – *Karen Bohnsack (FDEP) on behalf of Ilsa Kuffner (USGS)*
  - o Karen Bohnsack reminded attendees that during the last call there was discussion about how corals may be affected by disease even if they are not showing outward signs, and that precautions should be taken to ensure we are not unknowingly transmitting disease. Ilsa Kuffner (USGS) sent a non-bleach dive gear decontamination protocol that was adapted from the USGS zebra mussel decontamination protocol. This is still a draft awaiting final approval by the USGS Diving Program Board.

- Thierry Work noted that this protocol is still undergoing revisions.
- Karen Bohnsack asked that we table this discussion for now; the final version will be sent when available and included for discussion if necessary during a future call.

Working Group Updates

- NSF RAPID Grant Proposal
  - There are no updates on the status of the NSF RAPID Grant Proposal, although there are indications that it has been favorably reviewed.
- FKNMS Permitting Updates
  - Thanks to Joanne Delaney with FKNMS for support with permit revisions for additional sampling at Conch Reef.
- Sample Analysis Working Group Update: Microbiome Analysis – *Julie Meyer (UF)*
  - Karen Bohnsack reminded attendees that during the last 2 calls, Jan Landsberg (FWC) shared initial findings from the histological examination of coral tissue samples – including those obtained from FWC's sampling effort at Grecian Rocks last July. As a compliment to that, samples from Grecian Rocks were also preserved for molecular analysis. Julie Meyer from the University of Florida has been working on those samples.
  - Julie Myer noted that she received samples from 4 coral species (DLAB, MCAV, SSID, and CNAT), including both diseased and apparently healthy tissue from diseased corals, as well as healthy tissue from apparently unaffected corals. Overall, there does not appear to be a bloom of any pathogenic bacterium. There is no statistical difference in the microbiome communities between the completely healthy colonies versus the diseased areas; the microbiomes are also similar to those in previously analyzed healthy samples.
  - Julie cautioned that while this could mean that there is not a bacterial pathogen, it could also mean that we did not catch it at the right time, or that it is a low-level pathogen. It could also be that the surrounding healthy tissue in the sample swamped the bacterial pathogens from the diseased tissue (e.g., the signal was lost by bulk extraction).
  - The methods being used only target bacterial pathogens, which from this analysis do not seem to be there. A different method is required to detect viruses and funguses. Overall there is no evidence of a shift in the microbiomes.
  - Questions/Comments:
    - Karen Bohnsack inquired as to whether Julie could make a recommendation for how samples could be collected or analyzed differently that would improve the likelihood of detecting a pathogenic bacterium, if it is present.
      - Julie noted that although she used bulk extraction with the skeleton, the microbiomes present are similar to what is just in the mucus. There are probably more bacterial cells in the mucus compared to other layers, so surface samples may be sufficient for molecular analysis. Pairing molecular samples with histology is important to help localize where the tissue damage occurs and if there are associated cells.
    - Cindy Lewis noted that as part of the DCYL rescue in February 2017, Cheryl's team sampled at the disease line then immediately preserved ½ of each slice in a cryofreezer and Z-fix. In addition to this, tissue on 5 of these fragments was sampled ~4 cm above the active disease line and preserved in liquid nitrogen for proteomics. These parallel samples of the same fragments are important; these

methods can be shared with interested parties. In addition, actively dying fragments are being routinely preserved in Z-fix to capture the disease progression. These will be sent to Esther Peters for histology.

- Mauricio Rodriguez-Lanetty mentioned that Cindy was able to sample healthy and diseased DCYL colonies from Coffin Reef over the course of 4 or 5 months following the 2014 bleaching event. They were able to look at the microbiomes in the colonies that became diseased at different times; the data support Julie's findings that there is no clear microbial signature associated with white plague disease. One month after the 2014 thermal event, a species indicator analysis identified approximately 50 species of bacteria that seemed to be correlated with the white plague, however this signature was lost after a few months of new disease appearing on DCYL. There was no more evidence to support that bacteria were associated with the white plague disease. It is important to keep looking at these microbial compositions, but also to go beyond that to look at fungal and viral communities. An effort should be made to coordinate analyses of these other components. Their group is currently designing some primers to look at fungal communities.
    - Julie Meyer agreed that they should coordinate the microbial analyses, and noted that another option is to take a metagenomic approach to pick up viral components.
- Jan Landsberg inquired if the current primers being used would pick up intracellular bacteria such as chlamydia or rickettsia alleles?
    - Mauricio: The microbial communities being analyzed are those found in the mucus and tissue of the samples obtained. They have been able to look at the DNA of the symbiodinium, which is inside the host cell, so this extraction approach should be able to pick up intracellular bacteria. They can run analyses to see if the primers will pick up chlamydia or rickettsia.
    - Julie Meyer clarified that the primers being used target the V6 region; they use a combination of 4 primers that is designed to capture as many bacterial groups as possible. They have picked up some rickettsia, but need to double check if it would pick up chlamydia.
- For all future sampling efforts, Vanessa Brinkhuis requested that a portion of all molecular cores be sent to FWRI for storage. At least ½ of each core should be archived so as protocols are refined and there is more communication among the groups conducting the analyses, samples can be re-analyzed.
    - Val Paul noted that the November molecular samples are still in storage and a portion can be sent back for permanent storage. This will be coordinated off line.
    - Esther Peters also still has half of the SSID histology samples that will now be reanalyzed. Once the analyses are done, what is left can be sent back to FWRI to be catalogued.

Other Reef Issues

- Macroalgae Observations
    - Karen Bohnsack reminded the group about an outstanding question regarding a decline in observations of algae mats since Hurricane Matthew (early October) and cooler

weather, and summarized some follow-up conversations. The previous reports were of macro algae mats, which is distinguished from turf algae that is composed of short (8-10 mm tall) mostly upright filaments of multiples species. Algae mats are not uncommon, especially during extended calm periods when there are a lot of nutrients. C-OCEAN has not received reports of macro algae since the hurricane and cooler weather. Mote has seen macro algae blooms on the outer reefs in the lower Keys mostly during the early summer months.

- Other Issues:
    - Bill Precht reminded the group of his attached report on the status of disease affecting MCAV in Miami-Dade County. Regarding the inquiry about whether the disease has slowed down in the winter months, Bill noted that in August and September, disease prevalence was 35 – 50% among MCAV in the 5-mile area north of Government Cut (northern Miami-Dade County). More recent surveys in January and February showed that disease prevalence has declined to 15%. It is unknown if this decline is due to the cooler temperatures or because previously affected corals have now died, so there is a smaller population being affected. Meanwhile, it appears that the disease in the other coral species (besides MCAV) has run its course in this region.
    - Bill Precht also highlighted that there has not been much discussion regarding the impacts of disease on *Eusmilia fastigiata* (EFAS), and inquired about observations of live EFAS in southeast Florida or the Keys. Over the course of 250 dives between Fowey Rocks and Broward County, Bill only observed one live colony. Although the exact number of colonies that has been affected by this disease is unknown, it appears to have been highly impacted. The same is true for losses of DSTO and MMEA.
        - Sara Thanner (MDC) noted that they have seen a few live colonies at the Key Biscayne and Golden Beach artificial reef sites. They have not recently visited the natural reefs to document any EFAS there.
        - Trudy Ferraro (DEP FPS) mentioned that EFAS was present at 4 of the 12 sites surveyed within Pennekamp; unfortunately, 3 of those sites had active disease.
        - Amanda Bourque (NPS) also mentioned that the EFAS in BNP does not look good.
        - Cindy Lewis noted that they do not have quantitative data, but because EFAS is rare she will usually document it. This species was badly impacted by the 2014/2015 bleaching events; their current status is unknown.
        - Bill Precht clarified that he has seen more colonies in the Keys over the past 2 years, but hardly any in the Miami-Broward region, specifically along the middle and outer reef tracts.
    - Bill Precht noted that after the 2014 bleaching event he got a permit with Ester Peters, Les Kaufman and Steve Vollmer to sample ACER off of Northern Miami Beach. At the time, there was an active white band/rapid tissue loss outbreak. Esther did histology work and Bill's group has done ecology and distribution of the disease related to temperature. A paper with this information is forthcoming.

Wrap-Up and Adjourn

- Karen Bohnsack (DEP) provided a reminders and reviewed action items from the call:
    - The date of the next call is TBD; a calendar invite will be sent out.

        - We hope to have a presentation from Nicole Hays with Nova Southeastern University's Halmos College of Natural Sciences and Oceanography – regarding disease data from the 2016 SECREMP surveys. These data show the progression of the disease north from Miami-Dade to Broward to Palm Beach County through time (from 2014-2016) and the huge losses to species like MMEA and DSTO, as well as the more recent losses to MCAV that are continuing.
        - We will also explore the possibility of having a USGS-hosted webinar on the decision support process.
    - Karen will send a follow-up email with notes from today's call, as well as a final meeting summary from the last call. Attendees who are interested in participating in a separate discussion regarding the field trials of disease interventions will also be connected to have further discussions offline.