Florida Reef Tract Coral Disease Outbreak

Coordination Meeting #14
March 21, 2019
1:00pm – 3:00 pm

Meeting Summary

Attendees: Ana Toline, Jennifer Koss, Gena Parsons, Jenn Simms, Andy Bruckner, Lew Gramer, Chris Sinigalliano, Jennifer Moore, Alison Moulding, Bill Goodwin, Jocelyn Karazsia, Lonny Anderson, Cheryl Woodley, Curtis Gregg, Chris Kellogg, Caroline Rogers, Ilsa Kuffner, Katie Richgels, Debbie Santavy, Mel Parsons, Wade Lehman, Valerie Paul, Blake Ushijima, Xaymara Serrano, Joanna Walczak, Karen Bohnsack, Mark Knowles, Vladimir Kosmynin, Elliott Heart, Stephanie Schopmeyer, Lindsay Huebner, Rob Ruzicka, Jan Landsberg, Michelle Kerr, Yasu Kiryu, Kerry Maxwell, Lisa Gregg, Shelly Krueger, Sarah Thanner, Dave Gilliam, Cindy Lewis, Joshua Farmer, Julie Meyer, Ilze Berkins, Esther Peters, Jessica Miles, Erin Lipp, Brian Walker, Josh Voss, Ian Combs, Erin Shilling, Abigail Clark, Robert Brumbaugh, Michelle Pico, Jack Stamates, Andrew Ross, Bill Precht, Dan Clark, Leslie Henderson, Paul Fitzgerald, Beth Firchau, Allison Delaschmidt, Robin Garcia, Caitlin Brucker, Steve Blackburn, Amanda Bourke, Rebecca Ross, Jenna McNeal (68)

General Updates

Executive Coordination Team – *Maurizio Martinelli (Sea Grant)*

- A leadership body we call the Executive Coordination Team (ECT) has formed by representatives from four lead agencies: the Florida Department of Environmental Protection, the Florida Fish and Wildlife Conservation Commission, the National Oceanic and Atmospheric Administration, and the National Park Service. The five representatives are: Kevin Claridge, Director of DEP's Office of Resilience and Coastal Protection; Sarah Fangman, Superintendent of the FKNMS; Jennifer Koss, Director of NOAA's Coral Reef Conservation Program; Gil McRae, Director of FWC's Fish and Wildlife Research Institute; and Erik Stabenau, Acting Chief of Physical Sciences Branch of the South Florida Natural Resources Center at National Park Service. The ECT works to help track overall disease response progress, assess resource needs and overall response priorities, seek to identify potential sources for additional resources, and brief their respective agency leadership and, as appropriate, relevant legislators or regulators. It was recognized that a great strength of the ECT is to be able to support one another and use a collective, unified voice to push common priorities forward.

Disease Boundary – *Stephanie Schopmeyer (FWC)*

- In mid-January, multiple colonies of early susceptible species (MMEA, DSTO, CNAT, DLAB, and PSTR) were found at Eastern Dry Rocks and Rock Key (just west of Key West) with signs of SCTLD. Follow up surveys confirmed that this is where the disease margin is, though one colony of DCYL was found with tissue loss at Sand Key. As with some previous observations further up the reef tract, the disease appears to be delayed in midchannel reefs. By February, there was disease observed at the Newfound Harbor SPA. While there has been white plague observed in the Dry Tortugas National Park, there have been no reports of SCTLD.
- Q&A
    - L Anderson: Last week (~ 3/11) SCTLD was observed at Sand Key.
        - A Bruckner: Also observed SCTLD at Sand Key – primarily on MMEA but also on DSTO, PSTR, and DLAB.
        - S Schopmeyer: Thanks for the update, please share any coordinates to keep the recon maps updated.
    - E Peters: Are folks sampling MMEA? We haven't gotten many samples of the early susceptible specie, but they can provide important information.
        - V Paul: Agreed – they can be very useful for bacterial cultures.

        - J Landsberg: Agreed – would appreciate more MMEA specimens. There were some collected from Looe Key last year that have been processed. It might also be helpful to have specimens of the white plague affected corals for comparative data.
        - L Anderson: Can help collect these samples, if helpful!

Resources available on the FKNMS Portal & DEP Webpage – *Maurizio Martinelli (Sea Grant)*

- To introduce or remind folks, there are two resources for information on SCTLD: the FKNMS coral disease portal and the DEP coral disease page.
- The FKNMS disease portal is a public-facing site that includes the latest updates on the coral disease outbreak and response. It includes pages with information about the disease, summaries of the response activities, information on how citizens can contribute positively to the response, and materials for media such as photos, videos and other resources. In addition, there is a News section that provides quick updates from the many partners about the work that is ongoing.
    - In addition, if you are a partner, the portal is always open to providing updates on your behalf or, better yet, linking to products or other items from your institution. The portal is very well suited to provide a wider platform to share the information coming from all the partners – so, if you have an update or a story you want to share, please don't hesitate to get in touch with me or with Gena Parsons of FKNMS.
- The DEP coral disease page similarly includes some general information about the response but is a bit more focused on technical reports and summaries from our various calls and workshops and meetings. If folks are looking for research reports on a specific aspect of the disease – for example, you want to read the report that relates to some of the research projects you hear updates on this call – you can access those reports directly from the DEP page. The reports stretch back to 2015. I do want to note that, often, the FKNMS portal will include a quick summary of key reports with a link to the DEP page.
- Q&A
    - V Kosmynin: How is the information being shared with the public?
        - G Parsons: There is a coordinated outreach effort where partners are working together to share information. Each partner organization can have a Communications representative to provide information to and from their team. For example, Sea Grant outreach opportunities related to SCTLD are shared on the portal as well as via Sea Grant mechanisms. To note, no advertising dollars have been spent.

SCTLD Sessions at Conferences (Reef Futures, AMLC) – *Maurizio Martinelli (Sea Grant)*

- A session at the first Reef Futures Conference in early December focused on SCTLD. We heard presentations about some of the high-frequency monitoring work out of FWC, some of the pathogen isolation and probiotic work from the Smithsonian team, intervention experimentation, updates on the coral rescue and restoration trials plans, and some of the genetic sampling associated with the coral rescue effort. There was good attendance at the sessions and great engagement, including from some folks local to the Ocean Reef Club where the conference was held. In addition, the meeting of the Association of Marine Laboratories of the Caribbean will be held May 20-24 in Punta Cana in the Dominican Republic. One of the sessions is focused on bleaching and disease and a call to our partners was made for presentations on SCTLD. We have a few partners planning to attend and present on what we're dealing with here in Florida. This should be a good opportunity to engage with Caribbean partners.
- Q&A
    - V Paul: Julie Meyer also presented at ASLO meeting in Puerto Rico.
        - J Meyer: Indeed! We also heard about microbiome research and intervention experimentation, as well as on the initial disease observations in the Virgin Islands.
        - J Voss: Ian Combs also presented on 3D modelling.

<u>Research Updates</u>

High frequency intervention – *Elliott Hart (FWC)*

- Building off the work by Brian Walker and Karen Neely, this project sought to answer questions about whether intensive, high-frequency lesion treatments can change the trajectory of disease at a given site. Using sites established by Mote Marine Lab, this project identified and mapped all corals within three 10x10 plots near Marathon (offshore, midchannel, and inshore). Treatments were applied at a frequency of once or twice per week. Control plots were also established.
    - Any apparent or suspected disease was treated with amoxicillin-laden shea butter (5g amoxicillin to 40g shea butter), and then covered with modelling clay to keep the treatment in place. If the lesion breached the treatment, a trench was created and filled with the treatment.
    - Amputation was also considered where appropriate, but that only occurred once or twice.
- The team took a liberal approach to treatments – anything that looked odd was treated. Likely some treatments were not on SCTLD, especially for some species (e.g., SSID, SINT, SBOU). Treatments were first applied at the offshore site in October/November of 2018, but the control plots did not appear to have SCTLD. This suggested that treatments may not actually have been on SCTLD. Slide 11 shows the 'strange' species removed and the data shows more of what we would expect.
- Slide 12 shows mean percent living tissue falling at both control and intervention plots in offshore and midchannel sites. Slide 13 shows increasing treatment at all sites, including inshore reefs around March.
- Slide 14 shows corals that had been patched (i.e., does not include trenched corals). Although half of the corals were patched just once, nearly half required re-treatments elsewhere on the colony.
- With disease picking up at these sites, including at the inshore sites, we should have more data in the coming months.
- Q&A
    - L Anderson: Where are the sites specifically?
        - E Hart: A few km west of Looe Key. If names help, they are North Birthday (inshore), Wonderland (mid-channel), and Porky's (offshore).
        - L Anderson: Are these east of American Shoals?
        - E Hart: Yes, by a few km.
    - C Lewis: An observation from treating DCYL – repeat treatments have been successful. At KML, the treatments are with amoxicillin via base 2. As the leaching experimentation of base 2 suggested that all the amoxicillin is leached out in 3-4 days, the team has been applying 3 treatments 3 days apart. At the end of the 9-day treatment period, there is nearly a 100% success rate in a controlled setting.
        - V Paul: Very useful info. It is worth noting that in situ, the corals are going to keep getting exposed. For very susceptible corals, we don't know if that approach would work (but likely we would see higher success with slower progressing species like MCAV and OFAV). For example, there is the one DSTO in the dataset that needed 5 retreatments.
    - A Bruckner: This is a great study and will be very helpful in determining feasibility of these sorts of treatment approaches. A question specific to trenching – does trenching and applying an antibiotic treatment to the trench show success? If the trench is a in advance of the disease interface, have the antibiotics already leached out before the disease makes it to the treatment area?
        - C Lewis: At least in the laboratory, a treatment in advance of the lesion appeared effective within ~0.5cm of the application area (i.e., the disease stopped ~0.5cm of where the base 2 was applied). No trenching in this case, though. In the base 2, there is acetic acid which appears to cauterize the tissue/skeleton near the treatment which helped prevent algal growth on that surface for a week or two.
        - B Walker: Was the setup a recirculating system? Could amoxicillin be accumulating in the water?
        - C Lewis: Probably not, the system is flow-through, there should not be accumulation.
    - C Woodley: What was the rationale of applying the clay over the paste? One properties of clay is bind and hold things in place – could it be binding the antibiotics instead of releasing them?
        - C Lewis: Used modelling clay, which might have different properties than 'normal' clay. It is used to keep the treatment in place – often it helped keep it in place for 3-4 days.

        - E Hart: It was helpful in the field and found it stayed in place for longer periods. It will take more time to say definitively, but they may already have seen healthy coral tissue growing over the clay in a couple cases.

Pathogen identification & probiotic development – *Blake Ushijima (Smithsonian)*

- The first portion of this project aimed to identify and culture the bacterial pathogen responsible for SCTLD. To date, roughly 400 isolates from disease transmission have been cultured and tested. There have not been any conclusive or repeated results, suggesting we may be looking at opportunistic colonizers or secondary pathogens affecting corals with reduced immune response capabilities. That said, three families are highly represented: Vibrionaceae, Rhodobacteraceae, and Alteromonadaceae.
    - A metagenomics approach can also be pursued. Metagenomics, which would sample all the bacteria in a given sample, can help identify if we are dealing with an underlying viral infection.
- One thing to note – there is variation in disease progression through time. Sometimes affected colonies see lesions stop and start again – about half of the fragments brought in see disease cessation without treatments. This could confound field trials where we incorrectly attribute disease cessation to the treatments. In addition, some of the fragments from the Keys die very rapidly (some within 2-4 days).
- The samples that died very quickly had a high signal for *Vibrio coralliilyticus*, which is a known coral pathogen. *V. coralliilyticus* has strains that are resistant to both amoxicillin and penicillin – these have been difficult to eliminate in other land-based systems (e.g., oyster farms), so it could be a concern if many colonies with this bacterium are being brought in or if it becomes established in a coral nursery. The samples with the fastest tissue loss had the fastest tissue loss.
- The second portion of the project is to develop probiotic treatments. These probiotics are aimed to prevent disease transmission or slow/stop disease progression. This stems from the observation that some fragments used in transmission experiments are more resistant to the disease, and such resistance might be conferred by the associated bacterial community. These beneficial bacteria may confer benefits to the host or directly kill the pathogen.
    - Such treatments may be better than repeated antibiotic applications as (1) they can successfully colonize a coral for a long period of time (as opposed to just when an antibiotic is active), (2) presumably cheaper to produce the treatment (instead of constantly buying antibiotics).
- Inhibitory isolates (i.e., those with antibacterial properties) were collated in a characterized library to be tested. One probiotic strain appears to significantly slow or stop disease progression on diseased MCAV. This is summarized in the graphs on slide 9.
    - The library now has ~600 isolates. The plan is to optimize this treatment by determining what mixtures show low antagonism amongst the components but target a broad spectrum of bacteria. This allows the treatment to be broadly applicable with a lower probability of the pathogen(s) developing resistance.
- Q&A
    - C Lewis: This is very exciting! Do you put the probioitcs in a seawater bath with the coral? How do you apply it?
        - B Ushijima: At this stage, adding the probiotics directly to tank water. Right now, the focus is to screen the library for what might be effective and optimize the treatment. After more probiotic strains are identified can look at different delivery mechanisms.
    - C Lewis: Will you be trying to re-infect corals to see if the probiotic treatment persists?
        - B Ushijima: Yes, but that is further down the line. Some of the next experiments will be 'pre-treating' healthy corals to see if the probiotic mixtures can prevent disease transmission.
        - C Lewis: Very cool, good job!
    - B Walker: Are there plans to look at the microbiome after probiotic treatment?
        - B Ushijima: That's an interesting idea, but for now the focus is development of the treatment. Would be interesting to investigate down the road.

Microbiome investigations – *Stephanie Rosales (NOAA AOML)*

- Samples were collected from three distinct zones in the Lower Keys. (1) a 'vulnerable' site that was in advance of the disease margin and where disease was not present; (2) two 'epidemic' sites, where disease was active (one of which was opportunistic collection at Looe); and (3) one endemic site where disease had previously been present but where there was no active disease at the time of sampling. Within each zone, water, sediment, and tissue samples were collected. Samples from/around healthy colonies were collected at all sites, but samples from/around diseased colonies were only collected from epidemic sites.
    - Tissue samples can be binned according to: healthy tissue from healthy colony, healthy tissue from diseased colony, and diseased tissue from diseased colony.
    - A total of 265 samples were collected: 90 water (30 per zone), 90 sediment (30 per zone), and 85 tissue (15 in vulnerable, 15 in endemic, 58 in epidemic).
    - Three species were targeted: DSTO, DLAB, and SINT. MMEA were also sampled opportunistically at Looe Key.
- Following collection, the samples were taken to Mote and processed for DNA isolation, then they were sent to a sequencing facility where they were PCR amplified. The process utilized amplifies a relatively short strand of DNA that can be used for identification purposes but does not provide information about function. These 'amplicon sequence variants' (ASVs, which are unique sequences) are then input into a bioinformatics pipeline which assigns taxonomy using the SILVA database (a ribosomal RNA database). The output from this process can tell us what microbial taxa are present in the samples.
- The three sample types (water, sediment, tissue) had different microbial compositions and clustered according to type. When taking this a step further and looking at site status within each sample type:
    - Water samples clustered relatively strongly according to site status (i.e., water samples from vulnerable, epidemic, and endemic all clustered separately); these differences were significant.
    - Sediment samples were also clustered according to site status, but not as strongly as with water samples. Overlap was found in vulnerable and epidemic sites, but endemic sites clustered separately.
    - When looking at the data together, tissue samples did not cluster according to site status. However, when breaking out the Looe Key data (the opportunistic collections of MMEA) from those collected as part of the study, there is significant clustering/difference between site statuses. This suggests that disease is disrupting the microbiome of these corals.
- Two methods were used to determine if there were important taxa in these samples: a random forest method (i.e. a supervised machine learning method) that helps determine which ASVs best classify the three site statuses, and a core microbiome analysis that looks at ASVs present in 99% of samples from a given site status.
    - According to the random forest method, illustrated in slide 10, you can see a large change in relative abundance of ASVs from the order Rhodobacterales. You can also see ASVs from the order Rhizobiales in higher abundance in all disease samples except MMEA, and ASVs from the order Clostridiales present in all diseased samples.
    - According to the core microbiome analysis, illustrated on slide 11, you can see unique ASVs in healthy-healthy, disease-unaffected, and disease-disease tissue samples. Diseased tissue shows a spike in the family Rhodobacteraceae; they also show ASVs from the order Rhizobiales (the salmon bar identified by an arrow) that are only found in diseased samples.
- Water and sediment samples were explored to determine if they are reservoirs for disease. This step utilized a differential abundance analysis.
    - Sediment samples contained increased relative abundance of the genus *Nitrosopumilus* – a very common genus of archaea that is ubiquitous in marine ecosystems – in diseased samples. To note, *Nitrosopumilus maritimus* is an ammonia oxidizer.
    - Water samples contained increased relative abundance in *Rhodobacterales* and *Flavobacteriales* in diseased samples.

- Future directions include utilizing metagenomics for rRNA analysis and to assemble the *Rhodobacterales* genome, to sequence more samples for 16S rRNA analysis, and the collect samples from formerly healthy sites to determine if the same results are evident through time.
- Q&A
    - C Kellogg: How deeply did you sequence? What sequence numbers did you have per sample?
        - S Rosales: The ballpark was around 30,000 per sample.
        - C Kellogg: Sounds like plenty!
    - A Bruckner: Interesting findings! Where were the tissue samples collected from on the diseased colonies? For example, was is it sloughing tissue, immediately in front of the slough, or a portion of the disease line (capturing organisms in the interface)?
        - V Kosmynin: Same question about location of water and sediment samples.
        - L Huebner: For tissue samples, collections were along the lesion margin (i.e., right along the edge of tissue loss). Unaffected tissue was collected from the same colony but well away from the tissue margin. Water and sediment samples were collected randomly throughout each reef site – sediment samples were collected near coral colonies, and water samples were taken ~20cm above the substratum. At epidemic sites, samples were taken near diseased coral colonies.
        - C Woodley: For the tissue sampling – was it a superficial swab or was it collecting skeleton too? Some of the histology suggests that something might be coming from the skeletal side up.
        - L Huebner: Took blunt-tip syringes and scraped the surface of the tissue. This collected tissue, mucus, and sometimes bits of skeleton (often depending on species – e.g., DSTO often had bits of septa in the samples).
    - I Berzins: Are the organisms identified pathogenic?
        - S Rosales: Not sure. Something interesting is that some of them appear in other studies as potentially pathogenic (e.g., in white plague studies). It is hard to say if they are pathogens or just opportunistic colonizers. *Rhodobacterales* is known to be a good colonizer (it is often one of the first to create a biofilm on certain things), but not sure about the others. It was interesting that Rhizobiales were only found in diseased tissue. The two are often found together in the literature.
        - B Ushijima: It might be premature to classify *Rhodobacterales* as pathogenic – they are found thriving in many environments, and dying corals provide a rich environment. They may just grow quickly and be efficient about utilizing the available nutrients. For this reason, would err on the side of caution.
        - S Rosales: Good point, this data is just suggestive of bacteria that may be important in this disease or in a diseased environment, but not necessarily pathogens.
    - B Walker: What can we do with this information? We have this information plus what Blake Ushijima presented – can we make sense of both together? Are there methodological biases or similarities that we can narrow things down? Afraid that we are wearing two watches but don't know what time it is.
        - V Paul: To add, Julie Meyer has also been doing microbiome analyses. We most likely will not be able to identify a pathogen from microbiome analyses. They provide us useful insights and it is certainly not unexpected that the microbiome will shift, but it likely won't pinpoint what is causing the disease.
        - S Rosales: Have shared this information with Blake and suggested that there might be a benefit to enrich the identified bacteria, but the short sequences might not provide enough information to enhance the work. However, can use the 16S sequences and use them to see how similar they are to the ASVs to see if there is overlap. For example, seeing if the Vibrio present in the random forest analysis is the same as what Blake is finding.
        - B Ushijima: Agree. Can merge the 16s data but until we get more resolution we are mostly shooting in the dark. Some of the bacterial families are quite large and contain diverse range of

genera and species. But, moving forward, coordination between the groups would be beneficial. Would be good to pursue the metagenomic approaches.

- B Precht: Did anyone see *Thalassomonas loyana*. This bacterium has been associated in white plague in the Red Sea and was identified by in some analyses where the outbreak first began off Virginia Key.
  - S Rosales: Need more info on the family or order.
- V Paul: A reminder, the pathogen (if it even is a single pathogen) does not necessarily have to be the most abundant. It should probably be consistent between samples but not necessarily the most abundant.
  - S Rosales: Agreed. The core microbiome analysis aims to address that question – it looks at what bacteria were present in 99% of the samples. It does not consider abundance.

Caribbean Updates

Caribbean Cooperation Team & New Reports – *Maurizio Martinelli (Sea Grant)*

- Shifting gears outside of Florida, our Caribbean Cooperation Team is working to collaborate with and support however possible colleagues outside of Florida who are seeing signs of SCTLD. The team is growing, with members not only in Florida but in management agencies, NGOs, and others throughout the Caribbean. The hope is that the team can help provide information to *and* from other locations in the Caribbean, to help provide guidance where it is helpful and coordinate any regional activities related to SCTLD.
- As heard on the previous Coordination Call, a delegation of resource managers from Mexico visited Florida for a learning exchange to discuss what is being seen in Mexico and how they might respond. They joined Karen Neely in the field to observe and practice intervention and had the opportunity to meet with some of us at Reef Futures to talk through other related issues. They were in Florida for roughly a week and have since headed home and continued to monitor the spread of the disease and try their hand at intervention. They have been experimenting with some new compounds and have been sending information back to our Caribbean Cooperation Team on how those interventions are working. We'll keep the group updated on how that all is going on.
- A question that has cropped up multiple times is about terminology for the disease, especially as we start to see it outside Florida. As we do not yet have an etiological agent identified, we cannot make a true clinical diagnosis of the disease. However, we can use the term 'SCTLD' as a field diagnosis – to state that the gross clinical signs are the same, the same species are being impacted in the same order, tissue loss occurs on a similar time-scale, etc. Essentially the moniker says: it looks and is behaving exactly the same, even if we don't have a diagnostic tool to say with certainty this is the same. This is in line with previous terminology decisions made with other coral diseases where pathogens or etiological agents have not been identified and the nomenclature is more about gross disease signs.
- Q&A
  - I Berkins: From a veterinary perspective, is anyone looking into immune responses of the corals?
    - J Landsberg: There is not much being observed histologically, at least not in active lesions.

US Virgin Islands – *Leslie Henderson (DPNR)*

- As mentioned on a previous call, the US Virgin Islands have started to see signs of SCTLD. Currently, the disease seems to only be affecting St. Thomas but is actively spreading rapidly. It follows the same trends of temporal progression through species but MCAV seems to be closer to Florida's definition of 'highly susceptible.' This seems to be in line with the faster progression of Keys MCAV samples noted earlier in the call.
- Dr. Marilyn Brandt of University of the Virgin Islands has been mapping the spread and conducting some preliminary experimentation. She has also collected samples for microbial analyses and folks have applied for permits to conduct treatments.
- Folks are trying to spread the word through announcements and public meetings. There is much less capacity to respond in the Virgin Islands than in Florida, so getting folks involved will be key.
- Q&A

- V Paul: On a Disease Advisory Committee call, there was the suggestion that SCTLD might be showing up around ports or where ships dock. Is that the case? Flat Cay is also near the marine station – could is just be oversampling in this area?
    - L Henderson: The area where it first showed up is right around where large boats (e.g., cargo ships, oil tankers) anchor prior to entering port. It is also just downstream from Crown Bay Marina which gets a lot of traffic. It is difficult to say with certainty, but there does appear to be a correlation.
    - R Brumbaugh: Thank you for the information. Are ships discharging ballast in that area as they prepare to enter port?
    - L Henderson: They are not supposed to – ballast should be exchanged farther out and not over coral reef environments. However, enforcement of these things is always difficult.
    - R Brumbaugh: You should be able to access some of this information via ballast water management logs via Coast Guard.
    - J Walczak: Can help get that contact.
- L Henderson: A group from the USVI will be visiting Florida for a learning exchange in the coming months (similar to what was described with Mexico managers above). Hope to schedule meetings with folks during that time to learn as much as we can!

Wrap-up and Adjourn

- If you are interested in joining a Response Team, please don't hesitate to reach out to the relevant Team Lead or to M Martinelli. Info on the Teams and Leads can be found in the summary to Coordination Call #12.
- Standing reminder: please submit disease reports to SEAFAN (www.seafan.net) for the entire reef tract, including the Keys!