Coral Disease Workshop

Nova Southeastern University Oceanographic Center

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

Intervention Methodologies Workshop

November 6, 2017

Facilitator: Lisa Gregg

Attendees: Greta Aeby (University of Hawaii), Jeff Beal (FWC Marine/Estuarine Subsection), Ilze Berzins (Private Veterinarian), Karen Bohnsack (DEP FCO), Joanne Delaney (NOAA FKNMS), Sarah Fangman (NOAA FKNMS Superintendent), Paul Fitzgerald (Pinnacle Ecological), Dave Gilliam (Nova Southeastern University), Lisa Gregg (FWC), Charles Gregory (Private Aquatic Veterinarian), Kath Heym (Florida Aquarium), John Hunt (FWC FWRI), Kristi Kerrigan (DEP CRCP), Yasu Kiryu (FWC FWRI), Vladimir Kosmynin (DEP DWRM), Jan Landsberg (FWC FWRI), Cynthia Lewis (Keys Marine Lab), Lauri MacLaughlin (NOAA ONMS SEGOM), Maurizio Martinelli (NOAA Coral Management Fellowship), Margaret Miller (SECORE), Jennifer Moore (NOAA NMFS), Alison Moulding (NOAA NMFS), Erinn Muller (Mote Marine Lab), Karen Neely (Florida Keys Community College), Francisco Pagan (DEP CRCP), Valerie Paul (Smithsonian Institution), Dan Rothen (University of Miami), Joanna Walczak (DEP FCO), Brian Walker (Nova Southeastern University), Cheryl Woodley (NOAA NCCOS), Dana Wusinich-Mendez (NOAA CRCP), Roy Yanong (UF Tropical Aquaculture Lab), Aubree Zenone (DEP CRCP).

Welcome: Joanna Walczak thanked everyone for coming, and gave an overview of the ongoing multi-year coral disease event that led to the need for these workshops. A broad group of experts has been brought together since the disease event is widespread and shows no signs of slowing down; therefore, intervention, sampling, and monitoring strategies need to be explored. The group was previously given a document describing the draft etiology of the coral disease outbreak occurring along the Florida Reef Tract (FRT). Thank you was given to the Florida Legislature and Governor Scott who gave additional funding for disease response capacity in 2017, with a possibility of reoccurring DEP funding to address this issue going forward. Thanks were also given to Nova Southeastern University for hosting the workshop. The workshop schedule was described for each day. Lisa Gregg and Karen Bohnsack were introduced as the facilitator, and assistant for the first day. Lisa discussed the issues of permitting that are required for disease treatment, and asked for help from everyone (but specifically the veterinarians) on identifying potential intervention techniques and treatments that they would be comfortable supporting. Participants introduced themselves (see attendance list above).

Discussion: Coral Disease Intervention Treatment Methodologies:
Open discussion to identify what techniques have been used or should be explored. A summary of the methods discussed in the following live notes is available in Appendix A of this document.

- Shading and aspiration in-situ have been attempted on *Orbicella spp*. colonies afflicted with Yellow Band Disease (YBD) with no success [Erinn Muller]
- Trenching (deep trench into tissue a couple of centimeters wide) has also been attempted on YBD *Orbicella spp* with short term success up to 12-18 months. Initial trenching was performed in July, additional colonies were trenched every 4 months. No adverse effects were observed [Erinn M.].
    - Modelling/artisan clay, two-part epoxy, and cement can be used for filling in trenches and to fill holes left by sampling.
- Trenching was successful on a colony of *Dendrogyra cylindrus* afflicted with White Plague Disease (WP) in the Dry Tortugas; however, in some areas the disease stopped on its own.
- Further studies need to identify optimal trenching size, and proper location of trench.
- Hydrogen Peroxide treatment was successful at stopping Black Band Disease (BBD) in the lab [Erinn M.]
- Epoxy mixed with chemicals can be added to the barrier
    - Powdered chlorine mixed with epoxy was successful at arresting BBD on encrusting mound corals in Palmyra Atoll, Hawaii. The chlorine dissipates quickly and allows growth over the epoxy [Greta A.].
    - Chloramine-T and Bronopol (antifungal) are both antimicrobials used in aquaculture and can be applied in pastes [Roy Yanong]
    - Salt can also be used in an epoxy mixture, but the effects are unknown [Charles Gregory]
    - Chlorine and salt can be tested in flow-through aquariums, such as those in the Keys Marine Lab, with a UV sanitizer [Cynthia Lewis].
    - Mercurochrome can be added to epoxy [Vladimir Kosmynin].
    - See below for antibiotic discussion.

Action Item: Joanna W. will discuss chlorine and other treatment additives with EPA contacts.

- Removing diseased portions of colonies was successful in stopping lesion spread at the site of removal, but lesions would continue to appear in other parts of colonies in *Montipora capitata* [Greta A.].
- UV radiation has not yet been attempted but could be a good possible method of disease treatment [Margaret Miller].
- Antibiotics can also be a possible treatment either administered orally or through a vehicle (see epoxy discussion above) but face significant regulatory hurdles.
    - Medicated feed can be prescribed
    - Ampicillin (300 micrograms/ liter) is the most effective in lab treatment thus far [Cheryl Woodley].
    - Methyl cellulose as a vehicle for antibiotic transfer has been explored via dental paste [Cheryl W.]. However, the use of dental paste has not yet been well developed, and therefore epoxy should be used primarily while additional testing on dental paste continues.
    - Dilute Bayer insecticide dip and Neosporin were ineffective at treating a colony of *D. cylindrus* [Cheryl W.]
    - Amoxicillin and Kanamycin have been successful in treating *Montastraea cavernosa* and *Orbicella spp*. in aquaria. Antibiotics could also be delivered via bagging the colony or via spot treatment [Valerie Paul].
    - The microbial community will change when treatments (Chloramphenicol, iodine, freshwater dips, treatments for red bugs) are applied and would then return to

normal overtime when reintroduced to the original environment. This could occur with antibiotics as well [Ilze Berzins].
    - However, pretreatment of antibiotics to healthy corals allowed pathogens to infect the treated corals faster [Greta A.]
    - Testing must be conducted to determine the residency time of a given drug in the coral tissue after ex-situ treatment
    - Lab treatments are responsible for management and disposal of the drugs and all contaminated objects used in a study, and do not need veterinarian approval
- FDA considerations for "drug" use on corals:
    - The FDA considers any chemical that is used on an animal to be a drug [Roy Y.]. Disinfectants such as chlorine, salt, non-antibiotics, iodine, and freshwater are not considered drugs. Hydrogen peroxide, insecticides, clove oil, and Bayer dip are considered drugs.
    - FDA and FWC will need to be involved in allowing chemical use on corals, many of which have not been tested on corals yet.
    - Once the FDA approves the drug, the FWC (and when in the sanctuary, FKNMS) are in charge of permitting its use on corals in-situ.
    - The FDA is mainly focused on drug application as they relate to animals that we consume. A separate group (FDA Minor Use/Minor Species (MUMS)) works primarily on the animals that we don't consume.
    - Conversations with FDA MUMS regarding the *Dendrogyra* work have already been initiated and they are promising. MUMS will want to ensure that the treatment is being used only for the intended species and that it is not harmful to the coral.
    - NMFS should also be asked for permission for using specific treatments [Lisa Gregg]

Action Item: Jennifer M. (specifically on *Dendrogyra* intervention) and Roy Y. agreed to lead discussions with the FDA MUMS if specifics for treatments are identified.

- Small, anti-quorum sensing molecules that are used in human treatments could be used against bacterial pathogens in corals [Cheryl W.]
    - Carbon Dots used for bone treatments in humans may be applicable to corals [Cheryl W.]
    - Caution with anti-quorum sensing molecules was suggested because of the variability of bacterial pathways which could cause new issues for the corals [Valarie Paul].
- Probiotics have been successful in stopping disease infections when pretreated in the lab [Greta A.]
- Could also pre-treat disease by creating probiotics and stress hardening (hormesis) [Jan Landsberg].
- Florescence patterns of the corals were not able to identify disease resistance in corals, but Steve Vollmer has identified disease resistant genotypes in some corals. Neither are too important however in finding treatment options. [Erinn M.]
- Need a better understanding of "apparently/visibly healthy" tissues and the overall health of corals.
- Morphology of the corals is important in identifying possible treatment methods [Greta A.].
- A bagging method can quarantine diseased portions to protect apparently healthy regions of colonies.

- Paul Fitzgerald suggests a hypothetical method of imbedding a spike with a slow-release chemical treatment in corals at each disease site
- Myrrh is a natural compound used to heal wounds (it would be helpful to use natural products for easier permitting) [Cheryl W.]
- Natural oils could potentially remove coral parasites [Charles G.]
- Genetic modification and selective breeding strategies could be explored to prevent future outbreaks [Paul F.]
- In-situ culling of diseased corals at site and habitat scales should be considered [Cheryl W.]
- Formation of a genetic bank (cryogenics or other DNA samples) [Dan Rothen]
- Fire-breaks are better suited for large in-situ colonies, rather than fragments.
- Joanne Delaney feels that the intervention methods discussed here would require contextual approval, none are "absolute no's". The veterinarians present (Charles G., Kath Heym, and Roy Y.) confirm that they are comfortable with all discussed treatment methods that do not require FDA approval.

Discussion: Constraints Associated with Methodologies

The group was asked to identify the constrains of a treatment project like the one that has been formulated.

- Is funding and man-power feasible for scaling-up the above-mentioned treatment methodologies? The trenching concept is good but really labor intensive.
- Need to identify the facilities and tools necessary to test disease treatment methods.
    - For example, lack of specialized tools (such as a pneumatic dremel for underwater use) is a major constraint for trenching [Erinn M.]. When trenching, there would also need to be a tool that can suck up the coral pieces that are thrown into the water column while drilling [Valerie P.] so that they don't stay in the environment and potentially infect other colonies.
- There remains a lack of knowledge on the ideologies of coral disease, such as vectors, genetics, and other on-going stressors that can affect corals' overall health and disease transmission.
- The project needs to be effectively managed to ensure a unified effort and proper communication with contributors.
- There are current reef-wide disease assessments underway to identify locations of disease (shallow vs. deep reef).

Discussion: Feasibility of Methodologies

A 3-page summary of the current disease outbreak along the FRT was provided for this open discussion on applicable techniques.

- A matrix (Appendix B) of feasible intervention methods will act as a placeholder and will continue to be updated as appropriate to include:
    - Species
    - Currently feasible methods and methods that present hurdles for disease treatment
    - Need for regulatory approval (for both small and large-scale application)
    - Ease of implementation/ scalability
    - Treatment duration
    - Potential hazards to the host and ecosystem

Action Item: The matrix will be circulated for revision and input from workshop participants.

- Shading and aspiration were excluded as potential treatments for this particular outbreak due to general concerns over effectiveness.
- Trenching, lesion removal and back-fill, and fire break (depending on the coral morphology [Greta A.]) are good options for on-site treatments.
- Perhaps the best method is trenching corals while aspirating the flocculent, and using barriers without additives. Testing is needed to determine whether flocculent collection is necessary. These tests should be conducted in artificial areas or on isolated sand bottoms to avoid spreading the disease to apparently unaffected corals nearby.
- In addition to identifying methods for immediate use, ex-situ studies should be simultaneously conducted to identify methods that could be potentially scalable, methods that need improvement, and treatments that involve chemicals / drugs that require discussion with the FDA for scale-up.
    - These ex-situ experiments should be commenced as soon as possible [Margaret M.]
- Need to identify a convenient site for studying disease [Greta A.]

Discussion: Implementation of Trenching / Protocol Development
A hypothetical scenario where the trenching treatment would be used was discussed.

- Specific methods for trenching size, filler type, controls, site size, etc. were compiled into the live notes (Appendix B).
- Photos should document each time a treatment is applied and monitored (Photo mosaics are discussed as a possibility, but not to be implemented currently).
- Each treated coral should be mapped.
    - Roy Y. suggests that virology and microbiomes should be including in sampling protocol
- Colonies should be monitored one week after initial treatment, and then monthly.
- Two sites will be needed to study whether flocculent removal is necessary.

Action Item: Greta A. will create a standardized data sheet for data collection and procedures.

Appendix A: Coral Disease Intervention Treatment Methodologies Summary

Mechanical (in-situ and ex-situ) – All feasible from a regulatory standpoint

- Shading
  - More applicable for BBD
- Aspiration
  - More applicable for BBD
- Trenching/double-band firebreak
  - Cannot be lab tested because specimens are too small
  - Firebreaks ~2 cm
  - Should consider "back filling" the wound
  - Consider combining with aspiration of removed materials (test in a lab environment; collect the flocculent – test in lab and field experiments)
- Diseased tissue/lesion removal/amputation (of branch or pillar)
- Healthy tissue transplantation

Barriers

- Epoxy
- Cement
- Modeling clay
- Dental paste – ex or in-situ (Vets OK prescribing)
- Trenching

Delivery vehicles

- Epoxy
- Cement
- Modeling clay
- Dental paste
- Zooxanthellae
- Delivery vehicles used for humans in bone applications (nanoparticles, bone cement)
- Medicated feed
  - Depending on the medication and application, may need to be prescribed
  - Ex-situ or in-situ (depending on how applied/dosage)
- Fertilizer-type spikes

Mixed in to delivery vehicles

- Powdered chlorine
  - Not a drug; considered to be a disinfectant
  - Cannot be lab-tested in a closed system because it is toxic.
  - Can lab-test in an ex-situ flow-through system (with UV sterilization of effluent) prior to field application
- Antibiotics (Gentamicin, Paromomycin, Ampicillin, Amoxicillin, Kanamycin, Chloramphenicol, Enrofloxacin) – FDA regulated
- Salt (artificial sea salts, sodium chloride) – Not a drug
- Chloramine-T – FDA regulated
- Bronopol – FDA regulated

Application Methods

- In-situ bagging
- Immersions/Dips
- Dropper
- Topical application

- Injection
- Plastic lesion "casts" (injectable)
- Other antibiotic impregnated materials? (Izle to follow-up)

Other treatments

- UV-Laser radiative treatment
- Photo-activated compounds
- Topical applications (triple antibiotic)
- Hydrogen peroxide
    - FDA regulated as a drug (Peroxaid 35%)
- Insecticides (milbemycin oxime, bayer compound)
    - FDA regulated hormesis as a drug (for application in animals)
    - EPA regulated as a topical
- Immersions/dips (fresh water, Iodine) – ex-situ only
    - Not FDA regulated as drugs
- Mercurochrome (Vlad)
- Natural product compounds (probiotics, myrrh, organic oils – melaleuca, etc., garlic)
    - Case-specific (ask FDA)
- Phage therapy
- Immunotherapy
- Synthetic mucus
- Copper wire (as an antimicrobial barrier)
    - Not FDA regulated
    - Already approved for antifouling in boat paint

Preventative approaches (biological manipulations in healthy individuals)

- Vaccines/inoculation
- Selective breeding
- Environmental manipulations
- Stress hardening (hormesis)/immune priming
- Culling
- Genetic banks

Appendix B: Feasibility of Methodologies Matrix

Methodology	Regulatory Feasibility (Y - large or small scale/N)	Is it likely to be effective? (Y/N)	Easy to Implement (Y/N)	Potential Negative Impacts to Host (Y/N)	Potentially Complementary Methodology
Shading		N			
Aspiration		N			
Barriers					
Trenching/ Firebreak		Y	Y- Small scale (a few sites)	May add additional flocculent to environment (could be aspirated)	Aspiration Epoxy
Lesion removal					
Healthy tissue transplantation					