



These Q&A followed the webinar “Treatment and Intervention Approaches for Stony Coral Tissue Loss Disease” on May 19, 2020. This webinar was co-hosted by the U.S. National Oceanic and Atmospheric Administration (NOAA) on behalf of the Caribbean Cooperation Team of the Florida SCTL D Response Effort and the Reef Resilience Network.

The Q&A below include responses from the webinars four presenters with their initials included: Dr. Karen Neely from Nova Southeastern University (KN), Dr. Marilyn Brandt from the University of the Virgin Islands (MB), Mike Favero from Ocean Alchemists, LLC (MF), and Dr. Valerie Paul from the Smithsonian Institution (VP).

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### **General SCTL D Questions:**

In areas that have been heavily impacted by SCTL D and coral loss, have there been any observations on the ecosystem impacts and changes? For example, fish populations?

MB: This is an area of active research in the USVI.

How did you classify the susceptibility of coral species to the disease?

KN: For the case definition, this was largely a “gut-check.” Species that were seen diseased early-on in the infection stages were called “highly susceptible.” I don’t think this was ever quantified. However, Marilyn’s students have been doing this in a more quantified way and perhaps she can speak to their methods.

MB: Susceptibility can be defined many different ways, at the macro and micro level. In the USVI, we are trying to quantify first at the macro level and we are using similar metrics to what we used in a recent paper from my lab (Williams et al. 2020 Coral Reefs 39:27–31). Those metrics include some collected in laboratory experiments (time to infection after exposure, overall disease prevalence during an exposure experiment, and lesion progression rates) and some collected in the field (disease incidence rates, disease prevalence during a specific time frame, and lesion progression rates). We are also working with Amy Apprill at Woods Hole and Laura Mydlarz at University of Texas Arlington to look at the microbial and immunological characteristics of susceptibility.

Is there a way to positively ID SCTL D beyond the gross lesion morphology at this point?

KN: There is no confirmed diagnosis. In addition to lesion morphology, we highly encourage consideration of the species affected. For example, if lots of Orbicellas look sick but the Meandrinass are all fine, I would probably question whether it is SCTL D.



Does disease progression rate vary based on time of year? As in could summer/winter different water temps, amount of sunlight, additional changing conditions affect the rate of transmission?

KN: Val has better long-term monitoring data that she might be able to use to answer this. We have never measured disease progression rate across corals, but anecdotally we do not see differences between different seasons. That said, there are many anecdotal observations from Florida, USVI, and TCI in which disease lesions on corals that bleach during the summer show slowed or halted progression during/after the bleaching event. One of Marilyn's students has been quantifying this, so perhaps Marilyn can speak more to that.

MB: One of my graduate students followed corals through time during a bleaching event last fall and found that lesions slowed or halted on most corals during peak thermal stress, but it was species dependent. Paper hopefully to be submitted soon!

Is it really a single-pathogen disease? Or a combination of more factors?

KN: This is a topic of active research.

The photo of how much the disease has spread on St. Thomas is sobering! Can you share what is being done to prevent a similar situation on St. John?

MB: Unfortunately, at the time of me answering this question the disease has spread entirely around St. John. There are some bays that do not have the disease. There is now a dedicated strike team for St. John who are working in collaboration with the National Park Service to treat corals.

Around S. Thomas, how do you define an area of progression vs extent?

MB: Area of extent is where is what known to occur in the previous month. Area of progression was where it was newly seen in that month.

Have you seen this disease in octocorals?

KN: No.

Any recommendation for those that do not have SCTLD yet in their areas?

KN: I would suggest identifying what your goals and capacity are. In theory, a single infection event in a previously un-diseased region (such as disease first appearing via a shipping vessel or some other vector) could potentially be halted with rapid and thorough action. To efficiently do this, you would need to be prepared to act immediately (plans, permits, supplies, strike teams trained and on hand) and be very thorough (culling, treatment of ANY disease signs, perhaps limiting potential vectors in that region like recreational divers or boat traffic, etc.). This has never been tried, so it may or may not be effective. If such action isn't possible, I'd recommend thinking about what is possible and what your goals are; are there certain corals you want to save (species, sizes, locations, stakeholder interest could all be considered)? certain reefs to try to take a stand at? Further, we have no idea whether interventions will need to go on as long as



the disease is present in the environment... it is worth considering your capacity should that possibility come to fruition. There are lots of difficult questions to ask and hard decisions to make, but we do have many years and thousands of acres of data on what happens if you don't make those decisions and don't do anything.

MB: We developed a Disease Response Plan for the USVI territory that is currently being finalized which details all of our steps and I hope will be helpful for other regions. We plan to release that as widely as we can when it is finalized. We also have our website: [vicoraldisease.org](http://vicoraldisease.org) where we've provided as much information as we can on what we are doing about the disease. We are always happy to share our protocols, information, etc. so please feel free to reach out.

What opportunities exist for volunteers to provide assistance with this research?

MB: In the USVI you can report sightings of the disease and other unhealthy coral conditions on our website [vicoraldisease.org](http://vicoraldisease.org). We also have a VI Reef Response citizen science program that works with local dive shops to do dives at our coral nurseries where you can help us maintain corals for the future.

### **Treatment Methods:**

How difficult is it to apply the technique for branching corals?

KN: SCTLD is not known to affect Acroporids or Porites, and so there have been no applications to branching corals.

How much training is needed to 1) prepare the antibiotic and base paste, and 2) apply the antibiotic to corals in the field?

KN: Preparation is very easy and would require very little training (~10 minutes). Training on how to apply is variable, largely depending upon the skill of the divers and their ability to correctly identify SCTLD. Assuming they are good divers and can correctly identify where to do applications, we usually spend at least two dives mentoring new practitioners to point out helpful tips and tricks. As with most things, efficiency and improved application come with more practice.

What is the purity of the amoxicillin you're using in the USVI?

MB: We are currently using medical grade amoxicillin.

In St. Thomas, why there is no paired location for the treated site on the north-east side?

MB: The pairing of locations is not based on geography. It is based on when the disease shows up at the reef and the coral community at the sites. Therefore, the paired site for the northeast location is actually on the south side off of an offshore island.



Are surveys being done every week in St. Thomas, USVI? How much time is required in the field and with how many people in order to keep up with treatments?

MB: This work has been transferred to the three strike teams, one for each island. The strike team for St. Thomas goes out at least 1-2 days per week with 2-4 divers.

Can you explain ways that you manage the water outflow from the tanks with infected corals?

MB: The outflow is treated with UV exposure and then the outflow goes over a rocky area on land.

How were the putative pathogens exposed/administered to the healthy corals?

VP: In laboratory aquaria they were added in the water column. In the ocean, the corals were tented with plastic bags and the probiotics were applied in the bags for 2 hours.

How long does the probiotic live on the colony?

VP: We are still trying to answer that question.

How many species of coral have you isolated potential probiotics from, and are these always species specific?

VP: We have probiotics from 5 species, but one strain from *Montastrea cavernosa* is the farthest along in testing. We do not know how species specific they are yet.

To apply the antibiotic in an aquarium, is it better to use the largest amount of coral skeleton so that it impregnates to the deepest?

MF: The best treatment approach to date is to apply the Base2B paired with Amoxicillin to the skeletal coral in contact with the disease margin. Follow the perimeter of the margin with a thick bead of the Amoxicillin impregnated Base2b.

VP: In aquaria, antibiotics and probiotics can be added to the water.

### **Treatment Options:**

Is amoxicillin the only treatment for corals or are other treatments available?

KN: Many other treatments have been tried but so far none have been successful. These include: smothering with clay or epoxy, creating a trench and smothering with clay and epoxy, toothpaste with peroxide, chlorinated powder mixed with clay/epoxy/shear butter/QuickCrete, placebo Base2b, and various natural products (mostly trialed in Mexico). All were unsuccessful and none are recommended. Current trials include some other natural products (Marilyn is testing these in the USVI) and mixing chlorine into alternate treatments (Greta Aeby is testing these in the TCI).



What resin did the team in Florida use instead of epoxy? Have you ever tried or have in mind another innovative approach to manually to tackle in situ the situation related to SCTLD? (ex. water, sediment, or corals)

KN: I'm not quite sure I understand the question as related to "resin". We trialed chlorinated epoxy in three mediums: SplashZone two-part epoxy, Aves two-part epoxy, and modeling clay. The amoxicillin was also trialed in modeling clay and shea butter; modeling clay was entirely ineffective and shea butter was mostly ineffective. We currently use the product Base2b created by Ocean Alchemists. I'm also not sure I understand the question about manually tackling the situation. There may or may not be pathogen in sediment/water reservoirs. While there are people looking into this question, to my knowledge there are no plans on how to address these potential reservoirs.

What other antibiotics have been tried before Amoxicillin was chosen?

KN: Amoxicillin and ampicillin were both used in water-dosing of SCTLD-affected *Dendrogyra* in the lab. Cheryl Woodley and her team conducted this work and for water-dosing, preferred ampicillin since it dissolves more readily. However in creating a paste, amoxicillin was chosen because it DOESN'T dissolve as readily. I believe Val used kanamycin in laboratory trials, but perhaps she can speak more to that. Nothing but amoxicillin has ever been used in the field.

Could you deliver a drug using a method as the one used to stain corals with alizarine red?

KN: Maybe. It's never been tried.

VP: Yes, this is how we apply the probiotics in the field.

Is there another active compound beyond amoxicillin that could be used, such as a common natural compound such as a plant or a fruit?

VP: We are currently researching multiple avenues along this path in correlation with both Marilyn Brandt and Karen Neely. We have developed a fairly extensive library of tests conducted at this point without great success. However, the reasons for their current ineffectiveness is not known. For this reason, we continually retest some of the active compounds with more robust research in alternate formulations which target different coral tissue and delivery techniques. In short - yes - we absolutely can try alternatives to Amoxicillin. However, each time a new ingredient is attempted or altering the delivery tissue target with an existing compound a large amount of work must be undertaken to redevelop the accompanying formulations which is our true passion!

Based on results from the USVI on removing diseased portions from corals and having the non-diseased side with high survival, do you think a simple in situ "firebreak" without any treatment material would have a similar effect?

MB: I believe that has been tried by Brian Walker and Karen Neely's group in situ with low success. However, we will be trying it in the field with some of the moderately susceptible species in the near future.



Have there been any interventions that focus on altering the behavior of fishers to limit the spread of the disease?

KN: Not to my knowledge. Although presence of disease in Florida does not seem to relate to fishing pressure (it is present in marine reserves, areas of high fishing, and areas of low-moderate fishing).

If probiotic treatments become more successful, how could other territories and organizations begin to utilize these techniques? What would be a requirement? Is it possible for these probiotics to be mass produced and shipped? Would organizations require their own labs?

VP: We are looking at lyophilization methods to stabilize the probiotics. Each location has different permitting requirements and it would have to be determined if bacteria isolated in Florida can be applied to reefs elsewhere.

Given the favorable indications of the amoxicillin paste, how many other antibiotics can be incorporated and tried?

KN: Probably others. Things to consider would be efficacy, permit-ability, and current abundance. It is my understanding that some antibiotics are reserved strictly for humans to minimize development of resistance, while others are more accepted for things like agriculture and aquaculture because they are widespread and have been around for longer (ie, some resistance has already developed and there are alternatives for humans with that resistance).

What are the methods for feeding corals amoxicillin? Do you mix amoxicillin with coral food and target feed the diseased colonies? Has there been success with this treatment method?

KN: This is very preliminary. We have been able to successfully-target feed corals non-antibiotic food in the wild with minimal loss to the environment. We used a commercially-available food (Coral Frenzy 0.5mm) which is negatively buoyant. We put it into squirt bottles and fed corals at night. It took some practice, but we were able to get all species (OFAV, MCAV, PSTR, DLAB, CNAT, SSID) to feed while losing very very little food to the water column or other animals. We are now trying to figure out how to get the amoxicillin to adhere to the food pellets without them clumping or getting substantially larger. We are currently experimenting with a small amount of gelatin as an adherent, but are not yet to the point where we have tried feeding corals this again.

### **Treatment Effectiveness:**

Considering the amoxicillin treatment method, what are some key factors of the method that have enabled this to be successful?

KN: I think the only factor that has made this successful is the amoxicillin. Placebo treatments were unsuccessful, and many other treatment types were also unsuccessful.



Is there any relationship between the success of treatment and the prevalence of the disease? (Is more or less success in halting progression seen in an area with higher disease prevalence?)

KN: This is a great question that we are trying to look into. It seems that the success of treatment of individual lesions is the same regardless of the prevalence of the disease (in our case ~85-90%). However, the reinfection rate seems to vary based on species and site, and we have not yet teased these apart. For example, *Orbicella* seems to continually get re-infected while reinfection of brain corals and MCAVs seems to be much lower. In areas where disease has run through and wiped out many species, the majority of what we treat are *Orbicella*. So, our reinfection rates at those sites are very high, but that may be largely because of the species composition and very little to do with the site itself. We did have one site we were able to treat early in its infection stage, and that has responded notably well. However, that site is also the only isolated patch reef we have worked with and had a very different species composition than the other sites. So its lower reinfection rate could be because the species were different, or because it is an isolated reef, or because we were able to treat infection before it solidly established itself in the community. We just don't know.

Once the treatment is spread around the colony, if it recovers, could coral tissue grow back? Will the treatment be a barrier?

KN: The treatment is a paste that is ONLY spread on the active disease margin. It is not applied onto the whole colony. The paste dissolves over time (we almost never see it at one-month monitoring), and we have many instances where we have seen the healed colony growing back over the area where the treatment was applied. Brian Walker's group (which adds a firebreak with amoxicillin) has also seen healing over the firebreaks. In contrast, their firebreaks with chlorinated epoxy added do not seem to show regrowth/healing, even when disease is halted.

When treatment has been applied, does it only stop the disease to spread? Has there been observation of a recovery of the entire colony?

KN: I'm not sure I'm understanding this question, but yes, it is only applied directly to diseased lesions. We never apply prophylactically, although Val can maybe speak more to the potential of this with the probiotics.

VP: We have not gotten far enough with testing the probiotics in the field to answer this question, but in aquarium assays one probiotic we tested can prevent disease transmission to treated colonies.

None of the interventions can be considered practical, can they? If the cause of the disease hasn't been determined, will treatments will fail in the long term?

KN: I suppose this depends on the project goals. If you want to save certain corals, then we have presented viable options here. If you are trying to eliminate a pathogen throughout the Caribbean, then no, these will not achieve that goal. I think this question can be compared to human diseases; much illness is caused by poor nutrition, unclean water, and bad air quality, and it's critically important to improve all of those things. But you don't let everybody who gets



sick because of these things die in the meantime. Saving corals, identifying the pathogen source, and improving the marine environment are not mutually exclusive.

Does the amoxicillin/base 2B work systemically at the site? Has the tissue been tested at a various distance from the barrier?

KN: Based on reinfections, we do not think the amoxicillin spreads throughout the coral colony, but rather stays localized near the region of application. Josh Voss and his group have done some microbiome sampling at various distances from the treatment site and are awaiting results.

Is Dr. Walker's team naturally bacteria resistant, and is that what caused the healing of the planned probiotic treatment corals? Are there any possible explanations for the unexpected healing that we can learn from?

KN: VP can speak better to those specific corals and probably the mainland Florida region as a whole. I would note that the region that work has been done in has been affected by SCTLD since 2015. The region I work in is relatively naive to the disease. Any corals still alive in the mainland Florida region are likely pretty tough and hardy to have fought off infection for almost 5 years. Val has partnered on some comparison studies of MCAVs between the regions, but she can summarize that better than I can.

VP: The corals that are not as susceptible to the disease such as *Montastrea cavernosa* and possibly *Orbicella faveolata* can sometimes heal without intervention. We have seen this in our tagging sites where we have monitored some colonies for years.

### **Treatment Impacts:**

Among the mechanical, chemical and biological intervention options, have negative effects on corals or other organisms been detected?

KN: For mechanical intervention, Brian Walker's work has shown that firebreaks with amoxicillin heal over nicely while firebreaks with chlorinated epoxy do not. For amoxicillin, we have not visually seen any negative effects on the corals themselves or on other organisms.

Have the amoxicillin and other antibiotic negative effects on the health fishes around the reef?

KN: We have not seen any negative effects on fish or other organisms. In our case, we very rarely see fish biting the treatment; in the small number of cases they have been territorial damselfish or occasionally juvenile bluehead wrasse. In all observations, they have spit it out after biting it.

Were there any studies done on what effects amoxicillin has on other organisms that come into contact with it? Have any long-term studies been done on whether or not the dissolved amoxicillin settles and accumulates into the sediments?





KN: MF spoke to the degradation rates of amoxicillin that might impact accumulation, and I'll let him follow-up on that here. I would also suggest looking at the following references for background information and concerns:

1. Elizalde-Velazquez et al. 2016. Amoxicillin in the aquatic environment, its fate and environmental risk. <http://dx.doi.org/10.5772/62049>
2. Panditi. 2013. Assessment of the occurrence and potential risks of antibiotics and their metabolites in south Florida waters using liquid chromatography tandem mass spectrometry. DOI: 10.25148/etd.FI13080706
  - a. (this provides a literature review of toxicology studies of various antibiotics on algae, bacteria, and invertebrates in Table 18).

Have fishes been observed nipping the paste used to insulate the disease?

KN: Very rarely. In areas with territorial damselfish (particularly 3-spot), they sometimes nip at it to remove it (they spit it out). I have also very rarely seen juvenile bluehead wrasse take a nip at it; they also generally just do it once and spit it out.

Regarding the probiotics, are there plans to test for beneficial or adverse effects of a strain on species from which it did not originate? For example, what are effects of McH1-7 on *Orbicella faveolata*?

VP: We are testing this now in laboratory aquaria. The results seem to depend on the bacterial strain, and we will only proceed with ones that have no negative effects on non-target species. It would be nice to find bacteria that are effective on multiple species of corals.

### **Base2B:**

Is Base2b a commercial product?

MF: We limit Base2B availability to research groups actively combating SCTLD or investigating its use with other diseases. We are happy to supply and work with any and all scientist actively working on disease remediation. Manufacturing can be completed either on small or large scale and generally supply teams monthly with the ointment.

Where can you get Base2b? Is there any program that would provide treatment to leave in stock in case of an outbreak? I am asking this because we are in a remote island (Carriacou) and would be much better to be ready for when the diseases arrive than wait until it arrives and then try to get the treatment here, especially considering how fast the diseases act and that we have a health population of pillar corals.

MF: You can either go directly to [Oceanalchemists.com](http://Oceanalchemists.com) or reach us by email at [oceanalchemists@gmail.com](mailto:oceanalchemists@gmail.com). There currently is not a stock program in place for areas anticipating a disease outbreak. However, we would be happy to supply multiple jars of Base2B free of charge as a trial treatment. We can send this shipment to you where it can remain



refrigerated for an extended period time until its time need. If your region becomes infected and the Base2B proves successful we can start sending regular supplies immediately.

Can you elaborate more on Base2b?

MF: The best location to learn more about Base2B is to visit [OceanAlchemists.com](http://OceanAlchemists.com). The site supplies a detailed care information for the ointment, cost information, where to buy the active ingredient (Amoxicillin is sold separately), amount of infected lesion that may be treated per jar, along with many other helpful notes. If you would like to learn more beyond what is presented on the site, please feel free to reach out to me at 727-460-2989 or email me at [OceanAlchemists@gmail.com](mailto:OceanAlchemists@gmail.com). I love to speak in depth about research and relish any opportunity to do so!

Can we get the Base2b in Mexico? What is the cost?

MF: You sure can! The best place to learn more about pricing information is through the website [OceanAlchemists.com](http://OceanAlchemists.com) and follow the link to 'Coral Ointment Information'.

The cost is \$50/jar of ointment + shipping. Each jar contains 400g of ointment.

1 gram of ointment = 5 linear cms to treatment so 1 jar of ointment = 20 m (65.5ft) of treatment

Shipping to most Caribbean countries costs \$100-200 for 1-9 jars and \$200-\$300 for 10-16 jars. Request quote for larger quantities.

We will take care of all Customs Forms and Fees.

Amoxicillin must be purchased separately and costs \$116 per jar. This will be for 50gs of Amoxicillin (however they have excellent large quantity discounts) <https://phytotechlab.com/amoxicillin.html>

Syringes must be purchased separately from Amazon and cost \$30 for 50 syringes. We recommend having at least 9 syringes per diver on the strike team.

How should the amoxicillin and Base2b be stored and how long can it be stored in various conditions?

KN: Mike will answer this better and include the specs from Ocean Alchemists, which should obviously be followed if possible. However, I will note here though that we haven't always been able to follow those specs and haven't noticed adverse effects. For example, the specs advise keeping it refrigerated, but we haven't always been able to do that and, though we keep it out of direct sunlight, having to store at tropical temperatures hasn't resulted in changed performance.

What is the shelf life of Base2b? Many of us live in remote islands and shipping might not be to efficient.

MF: We are still in the research phase of shelf life investigation; however, I will confidently say it lasts a minimum of 1-2months and likely much longer if stored per suggestion (We



acknowledge refrigeration is not always possible). We have found a shipping method that is quite economical and would be capable of supplying Base2B on a monthly or bimonthly basis. We regularly ship to both British and US Virgin Islands, and most Caribbean islands successfully

within a few days. If the Base2B does go bad we would be happy to resupply fresh Base2B and be extremely flexible about cost, likely requiring little more than shipping and in some cases less.

Is it possible to combine the probiotic with Base2B? If not, why?

MF: Unfortunately, it is not possible to pair the probiotic treatment with Base2B. This is due to the interaction of several ingredients located within Base2B that would likely kill the probiotics as they are antibacterial. Note: Base2B on its own is not antibacterial enough to slow SCTLD without Amoxicillin. We are however in the process of developing some alternative treatment approaches to carry Amoxicillin along with other active compounds that could be paired with the probiotics with slight modification.

What is the half-life of amoxicillin/Base2b? Would reapplication be needed to those corals that are not showing as much success.

MF: Once the Amoxicillin is mixed into Base2B either in or out of water the Amoxicillin degrades at approximately 2% per day. This is the same rate of Amoxicillin degradation submerged in seawater in the absence of Base2B. Karen Neely would be your best point of contact for reapplication regimens. To my knowledge it is common that a single follow up treatment is conducted several weeks after the initial application.