Coral Microbes: Friends or Foes?

Dr. Blake Ushijima

Transcript

N.B. Each new timestamp denotes a change in slide in the video from which this transcript is taken unless otherwise noted. The speaker at each slide is Dr. Blake Ushijima unless otherwise noted.

00:00 – Karen Bohnsack: It is my pleasure to formally introduce Blake, and I apologize if I butcher some of these words, I'll do my best. Dr. Ushijima was born and raised in Honolulu, Hawaii and received a B.S. in Biology from the University of Hawaii at Manoa in 2010. During that time, he began working as an intern under Dr. Sean Callahan from the Department of Microbiology and Dr. Greta Aeby from the Hawaii Institute of Marine Biology on a newly formed project investigating disease in Kaneohe Bay, which you guys were just hearing stuff about. As an intern, he assisted in developing infection trials with the coral *montipora capitata,* and initial assessments of the bacterial populations associated with that coral. He continued working on coral disease after being accepted into the graduate program at the University of Hawaii, and his primary focus is pathogenesis and the pathogenic bacteria associated with coral diseases in Hawaii and Palmyra Atoll. He's currently focused on molecular mechanisms and virulence utilized by these coral pathogens, while also developing them as genetic systems. Dr. Ushijima received his PhD. in microbiology in summer 2016, congratulations, and he is currently a post-doctoral researcher at Oregon State University working in the lab with Claudia Hase investigating bacterial pathogens affecting species important to aquaculture, so I'll stop butchering your work and let you talk about it instead. Thank you.

1:28 – Dr. Greta Aeby: I'll let you do all the smart people stuff [laughter].

1:40 – Dr. Blake Ushijima: Alright, thank you for having me, like they said, my background is actually biogenetics, so I'm interested in the mechanisms used by pathogens with cells to cause disease, or that influence their virulence, but, this work by itself cannot really tell you everything you need to know about coral disease. That's why we're working with professionals like Dr. Aeby, who can actually put the things I find into larger context and give a better understanding of these diseases. So today, I'm going to talk a little bit about the coral associated with bacteria.

2:22 – Some of the main topics I really want to go over, just a quick introduction of the coral holobiont and the microbial population associated with it. And, I'll talk about pathogens and coral disease, so most of the coral pathogens have been bacteria, so a lot of my focus will be on bacteria. And I'm going to end with something kind of different, talking about the bacteria that are actually important for coral health and how that can influence these studies as well.

2:55 – So there are a few actually take-away messages, if you can understand this at the end of this talk. First, is not every coral disease is the same. You really can't generalize each coral disease, because it might be, it's caused by a different pathogen; each pathogen is different. Something to be very careful with is to not generalize microorganisms. So, there's more microorganisms in a cup of seawater than there are humans on the planet. Their diversity is

astounding. There are millions and billions possibly of different species, and they have such a huge effect on our ecosystem, but they, the mechanisms they use to do this are limited, theoretically. And the last thing I really wanted to drive home is not every bacterium is bad. Most bacterium are either harmless or are good for us; a very small percentage of them are pathogens, so there is a common thing of, "don't touch that there's germs," or "there's a million germs on the toilet seat," well, it's not just the amount, it's what's there, so, yes, a toilet seat may have more germs than your cell phone, but your cell phone might contain more things that cause you harm. Sorry, that really… [laughter].

4:38 – I really want to start with perspective. So, studying bacteria, it's kind of a different perspective than if I was studying whole coral colonies. So first, the coral polyp is the montastraea polyp, it's about 5,000 micrometers across. Zooxanthellae are about ten micrometers across, you can see these under a microscope easily. A lot of bacteria studied are about one micrometer in length. So these are actually, even if you look at hundred under the microscope, these are still very hard to see, and besides size, time is also very different when you're thinking about bacteria. So a montastraea colony takes decades to grow, zooxanthellae, they divide every four to six days under complete optimal conditions to multiply, you have the right medium for them to be growing, bacteria like these *vibrio*, some can grow, divide every eight minutes. So, every eight minutes you'll have a division, and then eight minutes later, each of those bacteria will divide, and they'll grow exponentially. So you can go from having a single bacterium to millions by the end of this talk [laughter]. It's a very different perspective, so not only is their generation time much different, but it also affects their evolution, and I'll get more into that later.

6:11 – So, first I really want to introduce coral microflora. So, coral, we look at the coral and see this awesome boulder or branching organism, but it actually consists of multiple different things.

6:27 – So, as most of you know, coral [is an] animal, we have the endosymbiotic zooxanthellae. There's also this mucous layer, which is what I was really interested in during my PhD. work.

6:44 – The reason for this is this very thick mucous layer that covers the coral surface is loaded with bacteria. There's some estimates on some coral species there can be up to 10^5 cells per millimeter of mucous. So this is a microscopy image from Garren and Azam, and so the zooxanthellae will further spread, and so this is the coral tissue right here, and this is the mucous layer that's been stained by a [inaudible], which we use to visualize bacteria, we use blue dots to visualize a bacterial cell. So this mucous layer is loaded with bacterial cells.

7:25 – So this coral will have all these bacteria on the mucous, but it's also found that they will contain a small amount, or a population of bacteria that's different from the seawater, but it also can be also thought of as this, there are changes that are made to this bacterial community depending on the environmental changes of the state of the host. So one of the mechanisms the corals use is constantly sloughing this mucous, so bacteria also have to keep up with this sloughing by the coral by dividing in the coral. So here's the same paper, all the bacteria being released from the sloughing mucous. And it's thought that there's just a constant exchange from the coral colony itself and the microorganisms inside. So it's believed that there is this set resident population where it's thought to be very important for the health of the coral, and this

transient population that's supposed to change depending on the environmental conditions. Now, there's a caveat to this, [which] is we don't know the mechanisms the coral uses to maintain its populations, and it's just now we're starting to realize how these populations of bacterium differ depending on the coral species due to the advancement of next generation sequencing. So generally, this is just generally though to be going on. There is a lot of papers being put out that really do support this with some coral species, but even with bacteria, it's really hard to generalize across all species.

9:17 – But what is the purpose of these things? So, a lot of these bacteria are thought of either as just mutualistic, they may be producing something for the coral, like micronutrients or something. Mutualistic, commensal, or parasitic, they could harm the coral if it's a too high population, or they could be doing nothing for the coral; they're just there. It's also believed that under normal circumstances on healthy coral that you don't really have one population dominating. One of the mechanism people think are going on here is that bacteria are fighting for nutrients, are just taking up space, or are producing antagonistic compounds like probiotics to actually keep down competitors, so it's kind of developing this network of antagonistic interactions so no one bacteria really dominates, and they find this a lot with the bacterial community sequencing [that] a lot of coral species actually have this diversity in healthy corals, while diseased corals, well, in some studies, they show diseased corals tend to sway more to one organism dominating. And later, I'm going to really go over this, it's really reminiscent of our own gastrointestinal tract. So, the coral is essentially a mucous membrane, and so is our [gastrointestinal tract], and a lot of research put into human health field on the bacteria that are associated with health field on the bacteria associated with heathy diet, poor diet, different health states of a human, and how that's reflected in bacteria as well. So, it's believed this can also be used as some kind of way to assess the health of the coral, because the coral with four microbial communities as you'll see later on could make it more susceptible to disease.

11:27 – So, in conclusion, the coral animal is not just the coral polyp, you have the algal symbiont and the microbial community all together form the coral biome, and when you have shifts in different directions, that's when you get into trouble. So, let's say, if the water gets too hot, that'll break down the symbiosis in the coral animal and algal symbiont. Also, bleaching; you have a nutrient or a runoff that will [affect] the microbe in the community allows for some damage to the other, and you have this imbalance, which [results in] coral disease.

12:08 – Alright, so, when you do have this imbalance, or you have introduction of a new microbial species that can cause disease that's when you end up with dysbiosis and you have pathogens that can attack the coral animal, so…

12:28 – So, like Greta already mentioned, there are several different disease signs: discoloration, growth anomalies, and tissue loss, so, this represents the marine responses, or the responses the coral animal can have to something like disease, which can also be very misleading to disease researchers because this leads to the idea that, well, there's a common misconception that if you have white plague, "oh, that's a bacterial disease" well, someone else will say, "no, that's a viral disease," it's like, well, they're kind of both, they could, in some cases, be caused by bacterium,

some caused by a virus, so, it's that cause the same disease signs. This really flows into my work on my PhD. where I worked on *vibrio coralliilyticus.*

13:28 – Greta mentioned this briefly. This is one of a fairly virulent pathogen we found in Hawaii, concurrent with other ones we'd found before.

13:40 – And, it caused montipora white syndrome. As you can see here, the destruction of all the tissue…

13:49 - …And there's some more pictures of the outbreak. My coworker Berman actually did a lot of community sequencing on the 2012 outbreak, and actually found this pathogen associated with the disease lesions during this 2012 outbreak, so we believe it is one of the major drivers for this outbreak.

14:16 – I really wanted to give you guys a kind of idea of what we've been dealing with for a more virulent pathogen. So, this is actually a laboratory fragment that was inoculated with [a disease] we worked with, and this [image] is 12 hours later, so you can see here, this one corner, the tissue has already been wiped clean, so that's just white skeleton. This video I'm about to show is actually over the next 48 hours and you can really see the progression of this disease. [Video begins] and you can see here, this is tissue loss, and it's essentially, the tissue will dissolve, and you have little bits of tissue and mucous coming off the coral fragment. But, you can see here, it's visible, the amount of bacteria that's in it, this particular pathogen will divide about every eleven minutes, so every eleven minutes, you have division, you can actually see the visible mucous on the bacteria being released by this disease.

15:30 – So, that leads me into the point I really wanted to make, is sort of, every lesion, every tissue loss lesion you see caused by coral reefs obviously a virulent pathogen, a coral, tissue loss, you say, "oh, white syndrome, caused by part of the mucous," well, I would have to refer you to examples of human medicine.

15:58 – Because, you can think of the term montipora white syndrome as say, pneumonia in a hospital picture. Well, you have this infection in the lungs, well, it's not only caused by a pathogen. It's caused by multiple different things. A general example is flu-like symptoms. Everything causes flu-like symptoms. Wrapped lungworm causes flu-like symptoms. Influenza causes flu-like symptoms, so it's the same [situation] with white syndrome.

16:33 – The same lesion can be caused by three different bacterial pathogens. There's probably more bacterial pathogens if you look at the state of Kaneohe Bay. And there's something I really, really want to express to you guys just because you have coral that has signs of white plague, it's not necessarily a bacterial infection, it's not necessarily a viral infection, it could even be a ciliate infection. It's something, the disconnect between the pathogens and terminology, so some of things I'm really trying to get across to people.

17:11 – But this also leads to another, something else I want to get across is the idea that any bacterium is bad for you, that any amount, like, a lot of bacteria will be bad for any coral. There's a common critique of our work is that when we dump any bacteria onto a coral, it's going to cause disease. We are putting a lot; ten to eight cells per millimeter is a lot, but I would

like to confirm, that's the lowest dose of the highest amount of infection, so it's not actually the minimum dose, it just makes the work I do much easier.

17:59 – But, not every bacterium is pathogenic. So this, *vibrio coralliilyticus*, this 48 hours later stripped the coral tissue bare, the skeleton. Now, if I take two more closely related strains, *vibrio neresis* and *vibrio cyclittrophicus*, based on their 16s rRNA gene, which is the commonly used tool to distinguish bacteria, these are actually very closely related to the *coralliilyticus*. I put the exact same amount of bacteria on this coral as I did this coral, and so we think, "okay, so we're dumping a whole bunch of vibrio, which has this reputation of, oh, vibrio are opportunistic pathogens, any amount of them on coral, it's going to cause disease." This is only just two examples. You can dump, there are non-pathogenic *vibrio*. You dump them on a coral, they will do nothing to the coral. Now, this goes even further, because bacteria are further down the strains, like a lot of human pathogens, there are pathogenic and non-pathogenic strains. A good example is *vibrio cholerae*. *Vibrio cholerae* is very prevalent in the environment. Now, if you have certain strains, like the 01, you have a chance of having an isolate that causes, that does cause cholera toxin and the necessary machinery that's attached to your gastrointestinal tract that causes this cholera that kills a million people every year. But in just staying a non-pathogenic strain of your cholera, nothing wrong with it. And this is what I'm finding with the *coralliilyticus*, so 80% of the coral exposed to this particular strain will get deep tissue loss. *Vibrio coralliilyticus* strain H1, this is isolated from a healthy fragment of *montipora capitata*. Its 16S rRNA gene sequence is 99% identical to [inaudible]. If you saw this in the bacterial community analysis, you would think this pathogen would be on this coral. *Vibrio coralliilyticus* strain RE22, this is an oyster pathogen, isolated in Oregon, a devasting oyster pathogen, kills, responsible for the death of billions of oyster larvae, 99% similar 16S r. If you saw this in the bacterial analysis you would think you'll have this pathogen right here. If I put these on coral, each one is not even virulent to montipora capitata. *Vibrio coralliilyticus* strain RE22 only had a 10% mortality rate, yet if you look at them in bacterial analysis, "oh, *vibrio coralliilyticus* is not a healthy coral," well, that's the very thing you have to be cautious about, is that for bacteria, it's not just a species, it's a strain that's important.

21:24 – Why is that? Why is it that we can have something that based on biogenetics is identical but one will cause disease and one won't?

21:35 – Well, a great example is a rise in superbugs nowadays. You have all these antibiotic-resistant bacteria popping up, you know, that's apparently killing millions of people.

21:40 – Well, it's through the spread of DNA and the virulence-associated genes associated with this. So, an important thing to realize about bacteria is unlike eukaryotes, which, that branch of the species is something that can mate with another member of its group and cause viable offspring, this actually doesn't apply to bacteria. You have bacteria in a completely different genera of exchanging different DNA, this has led to the rise of enteropathogenic coli, which will conjugate with things like salmonella and shigella and exchange virulence factors that create a pathogenic e. coli that kills quite a bit of people. Another neat trick bacteria have is transformation: the ability to just uptake DNA from the environment. Famous examples, a 1928 experiment from Frederick Griffith, is that he had *strep pyogenes* that causes strep throat, that

had these non-virulent strains mixed with the DNA of virulent strains, just the DNA, the bacteria will convert the non-virulent population to a virulent population. And lastly we have transduction. So this is e. coli, and we have a bacterial stage that infects the e. coli, so these viruses in fact will infect bacterial cells, but not every viral infection will kill these bacterial cells, some will actually transfer DNA from one bacterium to another. This is actually how we get cholera pandemics, because cholera is not actually pathogenic, both strains are not pathogenic in the environment. However, when you get one stage effects cholera and allow it to acquire these regulated external service structures that serve as molecular targets for a second stage, which grants the cholera the ability to produce cholera toxin. So you have this swapping of DNA that will convert non-virulent populations to virulent populations, and essentially, it will really be the same bacterium, the same stuff with a few extra genes and now you have a pathogen. So something to really be careful when thinking about bacterial analysis, is just because you found a particular species doesn't mean you actually found a pathogen, just a little caveat to be aware of.

24:35 – Just so you guys have some examples of virulence factors that can actually be transferred between these different bacterial species, things like antibiotic resistance genes, we call them superbugs nowadays; toxins, the reason why we have endopathogenic ?? control line is shigella actually conjugating its toxins over to non-pathogenic e. coli, now we have e. coli that cause severe infections. Secretion systems; so this goes along a lot with mucous-associated bacteria that don't actually have to penetrate in the coral cells to cause disease. Some carry what's called a Type three secretion system which is essentially a molecular needle on the surface of the bacterial cells. So this the bacterial membrane, and you see these little protrusions that are molecular needles which are all along the surface and the bacteria just comes in close contact with that eukaryotic cell, these needles are penetrating the cell and they allow that to inject toxins into the eukaryotic cell like coral cells, causing tissue losses. So, it never really even needs to invade into the host, just be in contact. Adhesins and invasins, they're different types of membrane molecules, and adherents for invasion of its host. This is one of the reasons why we do have a lot of *vibrios* that will be the same species, one will be pathogenic, one won't be pathogenic, that either hang on to their host or invade the host cells. And the most kind of worrying thing is pathogenicity islands, which are virus-trusted DNA that will carry any number of one of these virulence factors. A great example is vibrio parahaemolyticus, it's normally a human pathogen, right now it's actually wreaking havoc in Asia, it's completely destroying entire shrimp farms because this bacterium acquired [something] that produces a toxin that kills arthropods. No one expected this, no one expected the bacterium to cause such a problem for shrimp, everyone was worried about it causing [a problem] for humans, and now the shrimp industry, it's a billion-dollar industry, is especially worried, because entire pods, once one shrimp gets infected, it spreads like fire, and they essentially have to kill that entire pod.

27:30 – Now, see how all these different virulence factors, bacteria form incredibly fast, they produce these microneedles, well, why isn't every coral dead already? Well, there's multiple reasons. First is host variability; so, a lot of the pathogens recognize a specific receptor on the host as the molecular target based on just genetic variability between different individuals as an example, sneezing on somebody and they don't necessarily get sick, variability in molecular

targets. So, some bacterial pathogens come into contact with a host they would normally affect, but they just don't recognize it as a host. Or, the fact that it won't adhere [has to do with] scale. To a bacterium, a human would seem like an entire planet. In the aquarium, the tiny little shape of the aquarium would seem like a tsunami. Sometimes, it just won't adhere. The reaction on the coral surface is enough the push bacteria away, so just because you're not doing something with bacterium or pathogens, it won't necessarily cause an infection. Pre-existing conditions or stressors; some of the strains have been found to be more infectious when the host is heat-stressed. But, it's also environmental cues, on the flip-side of that, a lot of the virulence trackers will be activated at higher temperatures, so it's a combination of the fact that the pathogen, are you under the right conditions, are you in similar conditions to where you habitat is, are you allowing the pathogen to be pathogenic, or, I mean, if you have a completely healthy host, you're trying to infect it with a pathogen, you need to have some kind of stress that allows that infectious process. So, if you are looking for a pathogen to throw your bacteria on, and nothing happens, well, it's not necessarily the pathogen, it might not be the right conditions. And then vectors of fomite required for infection. So, I was reading what we found in the clean seawater, that might be hindering the waterborne transmissions. So, this is a microscopic image of, this is from an infected fragment, and this is a strain of *vibrio coralliilyticus*, expressed as yellow frontal protein (YFP). And you can see that it's from mucous and coral tissue; the zooxanthellae is red, and the yellow is all bacteria. So, we found from the diseased fragments in the laboratory that are releasing these microscopic little balls of mucous and tissue, and if you put these out, you'll have upwards of 10^{10} cells per millimeter of this mucous tissue flurry. So that's a hundred to a thousand times infectious dose required for this pathogen, and they're just floating around the water. Now, if you're putting that with corals in seawater, you're constantly having water change, will that effect be more transmission? Yes. Is this one of the ways that, more some pathogens you have an infectious dose? Is this one of the ways you can put an infectious dose in the environment, because hopefully there is not 10^8 or 10^9 cells of bacteria per millimeter, in, you know, bay water, terrifying, but yes, this is one of the ways you have the idea of infectious dose. There's some concern as well, a lot of the experiment are going to be seen as artificial because of having such a high amount of bacteria and you don't know enough about the ecology disease to actually deem that not true, because you could have something like these little tiny balls of mucous going through water, on land, out to the coral, now you have your infectious dosage. And then, host immunity, there's been an increasing amount of studies looking at the host immunity issue in coral. This actually goes back to environmental conditions, and it might not be an environmental stressor that reduces the immunity that normally would initiate disease in the environment, well, it's been found a lot of the times when you heat stress the coral, it's not able to defend against [a pathogen] as well. Is this happening when you're looking for your pathogen? Did you heat stress the coral? Was there a bleaching event before a coral disease outbreak? All these little things you have to keep in mind, not just the host put also the pathogen.

32:50 – Then now, I kind of want to change gears and kind of also reiterate that not every bacterium is bad. A lot of my research is looking at how bacterium actually help corals, and how that will actually influence disease studies.

33:09 – Like I said earlier, you have this group of transient and resident microflora associated with a heathy coral. Well, it is believed that they produce a lot of antagonistic compounds that can kill pathogenic bacteria. Several groups have already shown this. I'm actually finding the same thing that…

33:35 – … these bacteria were very reminiscent of our gut microflora, and most people's, if you've taken a course of antibiotics you know, that after a while that could result in antibiotic-associative diarrhea. Well, one of the reasons for this when you take drugs like antibiotics, [they] destroy the microflora in your gut. It could allow pathogens that were already there or are very small populations to invade and cause disease in your GI tract.

34:11 – Well, the same thing can happen to coral. So, if you treat coral with antibiotics, well, if you look at the animal, they look completely heathy, there's no bleaching, there's no, the [skeleton's] not out, there's no tissue loss. When you plate out the mucous, there is a destruction of the microbial community. You see here before antibiotic treatment, the amount of colony forming units (CFU) we get, after antibiotic treatment, it's severely decreased. However, when you douse this coral, this seemingly heathy coral with bacterial cultures, you find that actually they are more susceptible to infection and that also allows normally non-pathogenic strains to cause infection. Now, how does this affect disease studies? Well, normally, if you're putting these corals in seawater for along time, there's no input of the microbial communities, so you actually could have a loss of this community or a breakdown of the symbiosis. Well, now your corals are more susceptible to infection from a greater [amount] of microorganisms. Just something to keep in mind when you're keeping corals in artificial environments that destruction of the microflora could potentially skew the results of any pathogenesis studies.

35:37 – But now, why would these microflora be important? Like in the human gut, they can perform several functions; they give your cells specific vitamins and micronutrients. For the host, they could just be taking ups space to prevent pathogens from really expanding in population, or they could be producing antagonistic compounds that would inhibit the bacteria around them, so essentially, if any pathogen tries to colonize the microflora, or mucous of the coral, their growth would be inhibited or severely reduced. So my studies during my PhD. actually found this is true that can have isolates from heathy coral that do inhibit the growth of your pathogens like your *vibrio coralliilyticus*.

36:28 – So not only, so here's an isolate from a heathy coral and here's the pathogen. Most isolates don't actually have any growth spurt inhibitor activity in the lab, you see the pathogen grows right up to it. These two strains were actually isolated from coral fragments where I thought all of my experiments were somehow failing mysteriously, [I was] inoculating these corals with bacteria, and all of the sudden nothing was getting infected. I plated these corals out, the plates were then covered with purple and red bacteria which I'd never seen before, so this led me to believe, "well, something in the transient microflora changed, and now these corals are seemingly resistant to infection.

37:21 – And come to find out when I pre-inoculated this coral, other fragments, I had pre-inoculated them with these red-purple strains, have a severe reduction in virulence. So here, the

green line represents corals [inaudible]… the yellow line represents corals inoculated with the non-inventory, and the purple line represents [inaudible]. We have a reduction of about 60%.

37:55 – So, [we want] to have enough of a reduction in the isolate that we keep reduction in the virulence. So, we have these isolates that will come and go and depending on the environmental conditions they can completely dominate the coral microflora and actually prevent disease, one of the reasons we can have genetic, colony variation during these infection studies. But also something to keep in mind when doing infection studies is that there is, you are putting some kind of pressure on coral microflora, you could have a decrease in some of these protective isolates and also skew your results when you're doing infections.

38:42 – So some of the points I really want you to take home: different coral pathogens or stressors can cause similar disease signs. Like I said, over and over again, white plague, not necessarily a bacterial disease. White plague is just a term given to some kind of tissue loss on corals here, which is why we kind of need to think about how we're naming disease processes. Not every microorganism is capable of causing disease. So a lot of the studies talking about the amount of bacteria on your cell phone or toilet seat, well, it really depends on the type of microorganisms. And, non-virulent pathogens can become virulent, something to be aware of when doing bacterial community analysis, just because you see species that had been considered a pathogen before, doesn't necessarily mean you found a pathogen. And how important the microflora is for defense. When doing a live specimen manipulative experiment, you have to take that into account as well. Artificial environments you put these corals [in] you can't expect them to have an effect on the virulence of these pathogens and one of the reasons you can really look at your experimental set-up and make sure you have all the correct controls.

40:14 – With that, thank you for listening, if you have any questions, I'm happy to answer them now [applause].

40:25 – Attendee: I'm curious to know whether the fragments you kept in aquaria and that you affected with different forms of *vibrio*, how long did you keep those and observe them since you were looking for some sort of reaction in the first 48 hours or were you monitoring them for a set amount of time?

40:43 – Dr. Ushijima: So, we monitored them three weeks, we keep them in the tanks for two to three weeks just to make sure there's no hang stress or for some reason this bacterium takes longer to cause disease, so, but, it's always at least three weeks in the aquaria.

41:04 – Attendee: Did you ever try treating a diseased coral with [inaudible], did you dose them with beneficial bacteria?

41:14 – Dr. Ushijima: So, no, I haven't actually put them on diseased coral, but unfortunately for, once a disease starts, it's pretty much the next day there's nothing left, so it's really about timing, I would have to... I mean, even before the disease signs start, the bacterial load will increase about 10^7, 10^8 cells per millimeter of mucous, so it's kind of actually quite high, and then at a certain point there's, I would actually think there's a [low chance] you could actually stop it from really starting to kill the coral.

That’s an acute tissue loss disease-but, in a real world situation where’s the colony is larger, that’s not happening.

Dr. Aeby: Right, well, we do get a few tissue loss diseases in Hawaii [like this], but if you look at [inaudible]

42:22 – Dr. Ushijima: So, for this particular pathogen, I hadn’t tried it, but it is a very fast-moving disease, so you have to get the timing right, there’s a huge margin of error for that particular strain.

42:43 – Attendee: How frequently do you do water change when you’re doing these inoculation studies, and do you record or measure concentration of the pathogen after these water changes?

42:54 – So, normally for these studies, I would do a water change three days after inoculating, if the fragment was going to become diseased, actually would die before the first water change. But, I did take measurements looking at the amount of pathogens in the water on both infected and non-infected fragments, so the water actually, for this particular pathogen, stayed fairly consistent. Now, the infected fragments, they would have up to 10^{12} cells per millimeter of mucous tissue flurry. Uninfected fragments actually very interesting, they’ll either be almost completely devoid of any traces of pathogen, [inaudible].

43:52 – Dr. Aeby: With that said, bacteria growth is very rapid, so it really depends on the bacteria.

44:04 – Attendee: So, in the experiment, did you check the water quality from the bacteria? So, what I’m assuming is, sometimes the bacteria [inaudible].

44:24 – Dr. Ushijima: Well, for these particular bacteria, we don’t find them growing in water. The population stays relatively consistent for the first few days and then it actually starts to drop, there isn’t really an expansion of the bacteria in the water.

44:40 – Dr. Aeby: Also, Tanesh, that’s why we use the control bacteria to look at that effect there.

44:52 – Attendee: So for the strains of bacteria with the potential for protective effect, have you considered doing a pre-inoculation to see if that kind of work as a shield?

45:06 – So that was the experiment; so all the corals are pre-inoculated with the potentially protective bacteria and then we move them to a new tank and they’re inoculated with the pathogen.

45:18 – Attendee: And I totally understand, but the idea is how long will this last and what is the potential that it would not take.

45:27 – Dr. Ushijima: Yeah, I haven’t done those studies yet. Last time I was there I was kind of limited on the amount of fragments so I need to… time, and I’m using a lot of coral fragments. And really, for the point of that experiment really is just [to] show if there were bacteria from the healthy mucous that would, that could produce some coral fragments with disease. There’s, our

collaborators are looking more into actually doing that exact experiment with oysters and other shellfish because they are doing something for industry, so…

46:14 – Attendee: Very good. Have you though about doing any field inoculations, have you thought about foreign events? [laughter]

46:22 – Dr. Ushijima: Um, no… Dr. Aeby: Yeah, regulations are real tight so we had to be real careful. So, our idea is to try to develop some things under these conditions and then slowly and think about whether we need to do that in the field. That's why, originally the one with toxins was not toxic. We had to add the chlorine because it wasn't working and then it worked. But then you add the bacteria into the environment, so you, [it's already there]. And that may be, in the future, where we're headed, where it is naturally there and as coral biologists, we do an [experiment] by putting a bag there… and leaving if for twelve hours and not putting it off. And I've always thought about that, and thought maybe that's where we're headed someday. So, good ideas.

47:17 – Dr. Ushijima: I was like to include that yes, it's already there, but we're actually adding a very large amount. You really don't know the effects it could have on localized area, like for *vibrio coralliilyticus*, we know low amount won't do anything to the coral, we know a high amount will kill the coral. Coral aren't the only organism, on the reef, so I really don't know adding a huge amount of bacteria that protects coral will affect, you know, the shrimp around it or other animals. Dr. Aeby: You could try and see. We need a lot more research first to look at, does it affect other organisms, I mean that's work that can be done, right? I mean, again, coral disease research is a step-by-step understanding and [inaudible] methods of treatment.

48:18 – Attendee: Well I was just wondering if, I mean, I had a question, it might have sort of been answered but I want to bring up the point anyway, can you, I guess, for lack of a better word, vaccinate out-plants? So, before they go, give them a little bit of an advantage before they're out there, so maybe, like, once they're in the environment, it changes to a more natural level, but at least they have that head start with the probiotics or the vaccination. Is that a possibility?

48:51 – Dr. Ushijima: It's possible, so, are you talking about actual vaccinations, or pre-colonization with probiotic bacteria? Attendee: A pre-colonization but in an out-plant effort so that you're not risking the whole environment, you're only risking the one coral that you're maybe planting out.

49:06 – Dr. Aeby: So, I'll explain since Blake is not the coral person here. So what you're doing now is, because the acroporas have gone downhill so much, what we're having them do is grow up in nurseries, then put them back out on the reef to have them try to regrow on their reefs, so what she's saying is, "huh, first off, you'd have to know what probiotics are effective, what pathogens are out there, what are the probiotics that protect from which pathogens," and that would all have to be worked out in the lab to figure it out. Then you might think about populating it with particular probiotics that are effective against the pathogens that they're vulnerable to. And then, you'd have to do further studies, you'd have to think about, "okay, it's populated by that probiotic, does that microbial community stay stable through time, or is it

switched back?" Right, all those questions have to be answered before you can make your management decisions, those are really good questions that should be addressed.

50:08 – Dr. Ushijima: I mean, it is a possible thing we could do, but… Dr. Aeby: We've got to do the research. Dr. Ushijima: Yeah.

50:22 – Attendee: So, if I understand correctly, it seems like there are multiple causes of diseases, and you don't know which one is going to happen or which particular effect it is. But, let's say, the instance we saw earlier, well, bacteria are in the water, and then you know that it could be a bunch of different types. How do you manage that, what do you do? Okay, we're studying this really minute detail to understand is it the type of bacteria or this kind, you've got bacteria of that kind, maybe this one, it's in water, okay. And you that the doses change depending on water conditions and stuff like that. So, in terms of management, is this information as important as is being able to convey this information even though it's different species, different environment. Two, the folks that are making decisions about water management and the potential sources of sedimentation or turbidity and those types of things, to not have to put in millions of dollars and ten years of time to understand the specific organisms causing the issue here, but to use the models elsewhere to make decision for management.

52:12 – Dr. Aeby: So, you could make some, there's some generalizations you can do. You can say that corals living in an optimal environment are going to more resistant to disease. That's a generalized statement, but you could say it. So, right now, you could make management decisions, [regardless] of what was causing that disease to clean up the water and replenish the fish stock, so the balance of the ecosystem is put back. That will help maintain the populations and creates a more resilient population without doing anything else, but we're only at that point where we can say those things because there's research that has been done that otherwise we'd sit there going, "I don't know." So, I'm a big proponent, keep the research [going], in the meantime, let's go ahead and start taking some of these generalized steps from a management point of view of cleaning up our environment the best we can so we're [inaudible] because we can't do one or the other. We can't just do research and we can't do management actions that's not based on science, that's not going to be a very good thing.

53:19 – Attendee: Well, it would be based on science depending on using the model from somewhere.

53:23 – Attendee: Yeah, the issue there is just time, right, how long you wait before you make a change, right?

53:31 – Dr. Aeby: Well, like I said, you should be taking management actions now to clean up the environment. It's the best thing you could, I think of coral disease as analogous to human disease back when we were putting all our sewage out on the street and we were wondering why everyone was dying. Because people don't understand, right, we didn't know that. You wanted it out of your house, and plop, and we had all sorts of disease outbreaks, and once we started understanding the links between the sewage contamination and these invisible things that they finally figured out were germs, right, germ theory, then we started actually taking action. And we're in the same place with the coral, where, you're right, here was our system, we had humans

and sewage, now we've got corals and sewage. Right, so should we be taking actions when we understand that we should be, yeah, [look at] the sewage system in the Keys, the sewage system is getting switched over. So, they are starting to say, "yeah, we know these are problems that need to be taken care of that will help the corals." But again, in the meantime, if you don't know who your pathogens are, you don't know how they're transmitted, and you don't know which corals are susceptible, it makes it, other than a general thing, it can't be easily managed.

54:55 – Dr. Ushijima: A lot of the specifics of the things for the bacteria, it's actually more for the fine-tuning after, so you can make some generalizations and have some immediate action, but, further down the line, if you want to look at the specific pathogens that when you start looking at the more specific aspects of the bacterium.

55:18 – Dr. Aeby: Like the acropora that shows it bleaching in, that's when you go to your legislators and say, "look we've got the science now that links this with this and that needs to be addressed now."

55:37 – Attendee: We were talking earlier about these particulates. We have certainly seen those over the years with dredge projects, you get sediment in the corals and the you get a lesion and then black band and so on. What's the best studies out that show the sediment and the spread of the diseases, is there? I mean, I know of a study that's out in Australia now, I mean, what's the best stuff that you know, and how big of a factor is this, personally, from what I know it seems to be when we throw the particulate and the sediment in the mix and then mix it with the bacteria, it was a study, I believe it was on *cervicornis*, they were dosing corals in tanks, they weren't all getting the disease, [then] there was sediment in the tanks as well, a lot more of those corals got disease, can you speak on that?

56:36 – Dr. Aeby: Does anybody know that? I'm not familiar with that study. If you… nobody? So maybe we could talk afterwards and you can… We could look into that, it could be interesting, it wouldn't surprise me. Attendee: Especially with the dredging project coming up, I'm scared to death of what's going to happen.

57:02 – Attendee: One thing is the sediment, another is the contaminants that are buried in the sediment. So we're going down four or five feet, it's decades, the composition that's been degrading and all these different things. So, it's sediment but also something else dug up.

57:27 – Attendee: I'm not as familiar with the [research], I know the citation, but I'm not familiar… Dr. Aeby: That's about all we have out there, I mean, we're just now getting to the point where we can make things, you know, if you don't know what the pathogens are, we were at such a low-level of understanding, like when I did my post-doc fifteen years ago, it was like, clueless, I mean, we're just now getting to the point where we can, we can do some more focused research on this in there.

58:02 – Dr. Ushijima: I mean, it might not hurt to look at some human medicine, for example, bacteria coming out of sediment, cholera, in the winter months, it's usually within sediment, that attaches to the copepods to get back into the water column, green-eating amoeba [is] growing in the sediment, then it gets in your ear or your nose and gets in your brain. Dr. Aeby: Wait, where

is this [laughter]? Dr. Ushijima: Yeah, it's in freshwater lakes. I don't want to say anything [laughter].

58:54 – [Inaudible question]

59:20 – Attendee: I wanted to get back to the acropora study, I don't quite recall that one, but there's a long line of research on the effect of sediment on corals and we do know that the sediment falling on the coral surface is trapped in the mucous and it can't be removed also by the ciliary action as well, then that sediment sitting there is going to cause damage to the coral tissue, the bacteria are attracted to and then you have additional problems, and so, there's a number of studies that are showing that, and then you look at some of the newer work that's come out on dredging issues that are affecting corals, I think we can all agree that, yes there are problems.

1:00:18 – Attendee: I just want to add, too, that during the dredging project, [two people] brought a paper which was very outdated at the time but we were able to actually use that to adjust and, I guess, sort of set the bar, lower, higher for the NPU level that was allowed before we had to a shutdown, so I just want to encourage you guys, I have a whole list of things that we did to try to address, reduce the impact from the dredge project that I can share with you as far we had permit conditions but get some of these papers, and start bringing them to the attention of some of the agency people you work with, like the SEFCRI, and others that were involved in these planning processes, and you know, this was again a sanctuary, this was this massive inter-agency leading that started in 2001 and didn't finish until 2003 and we were constantly, you know, we had a very receptive audience, we had the Navy who was willing to be a good partner to the sanctuary and with their constraints, they brought in the Army Corps of Engineers and other folks as well, so we had kind of a unique scenario.

1:01:40 – Attendee: That project was relatively short in duration. Other attendee: Well, it was 2003 to 2007. And it didn't go without problems. But, I do encourage you to look at some of these papers, and I know there's been more recent work done, like some of the [work] on sanitation, and bring that to the table when you're working on projects with the agencies.

1:02:17 – Karen Bohnsack: And I actually have one more question from the webinar, so I'll try not to butcher this one. Do you know the difference in percent identify in the 16S rRNA between different vibrio coralliilyticus strains used in your experiment?

1:02:36 – Dr. Ushijima: Yeah, they're all about 99% identical, the difference is between four and fourteen differences over the 1500 size gene. So out of the 1500 types of gene, there were four to fourteen differences, so 99%. I mean, we look at the sequence, and we're going to say, "okay, that's the same matchup.

1:03:16 – Attendee: So, are there different activations then, things being turned on or turned off that differentiate them further in terms of virulence?

1:03:27 – Dr. Ushijima: So, yeah, so they're multiple things. What I'm finding is, so, some of the strains, they respond differently to temperatures, so, for example, the strain in Hawaii actually has a steady amount of virulence depending on if it's cold or warm water, which is probably one of the reasons we have outbreaks in the winter in Hawaii, while the strain from Palmyra and the

strain from the Indo-Pacific, they all have, they're actually more virulent at a higher temperature, they found a lot of genes associated with virulence are upregulated at a higher temperature. Also, a lot of the genomes of these things have been sequenced, and they defer, there's insertions in the lesions of hundreds of thousands of [inaudible] there's hundreds of genes where, one strain may have it, one strain may not, but for the third strain, it could be a completely different set of genes, because when they're doing biogenetics, they're based on one sequence of a 16S r, it's a single gene out of the 5000 or so genes in each of these strains, so you can see how, based on biogenetics they might be the same, but if you look at the entire genome, they can be very different.

1:05:00 – [Inaudible question]

1:05:24 – Dr. Aeby: So, during the, we have done the histology on this tissue-loss diseases from the wild, and we haven't seen any fungal, if you've got an aquatic edge, you're going to have a bunch of stuff in there, but nothing that's really consistent among those lesions, so it's going to point toward an organism. The only time we've found fungal infections is causing problems with dark spots, with hypermycosis, and that was discovered through histology, not the tissue loss. And, you know, we've done sequencing and all that stuff… but that is a good thing in Florida, that you guys are working together.