Laura Marangoni: So today we're going to show you how we extract the tissue from corals using the airbrush, that is usually used by painters, like to spray paint... so we adapted the whole system to detach the tissue from corals from their skeletons...

Leila Nilipour: An airbrush is a tool that uses compressed air to spray paint in a controlled manner. It is usually employed by artists. However, you just heard a scientific adaptation of it. Instead of paint, Laura Marangoni is using filtered sea water to spray a piece of stony coral. The water pressure helps remove the coral tissue from the hard coral skeleton, and this tissue can then be used for experiments.

LM: Here I'm going to measure proteins in coral tissue, because this is a good way to find out if they're healthy or not.

LN: Laura is a Brazilian coral physiologist, and she wants to find out whether certain coral communities in the Panamanian Pacific have developed immunity to climate change. Measuring the protein in the coral tissue is just one of the methods she's using to answer this question. But what is a coral physiologist in the first place, and what is this climate change immunity about?

LN: Welcome to Biodiversa, the podcast of the Smithsonian Tropical Research Institute in Panama, also known as STRI. My name is Leila Nilipour and I am a science journalist. In this episode, we will join Laura to learn about her role in the ROHR reef resilience project, an ambitious set of experiments in the Tropical Eastern Pacific aiming to uncover how corals cope with environmental change, information that could potentially aid threatened corals around the world.

Andrew, is that the start?

733 is the first one, 743 is the last one... and then you have the extra, 77 something.

What's the name of this spot? Canal Afuera...

So they're helping you now. Yeah, they're finding the transect. Right this minute, they're looking for the beginning and end of the transect. And they'll put a buoy in each.

LN: It's a beautiful sunny day and we're on a boat in the middle of Coiba National Park, a marine reserve within the Gulf of Chiriquí in the Panamanian Pacific. And although I've been to many places in Panama before, none of them have felt quite like this. So pristine, so full of life everywhere I look. In fact, it is the first time I can hear the crackling sound of coral reefs while sitting on a boat, a sign that the reef is full of life and, therefore,

healthy. We're joining Laura and Sean Connolly, a staff scientist at STRI, in their field work. They're studying the coral communities growing below us near Canales de Afuera, an island about 9 miles from Coiba island, the largest one in the marine reserve. Although pollution and overfishing are very immediate and local threats to coral reefs everywhere, Laura and Sean are interested in figuring out how these reefs will cope with the global, and often, intangible, threat of climate change.

Sean Connolly: So we'll do the coral transects, we'll do the photomosaics and the coral fragment sampling, and then after that, the fish team will go to a different site to do fish surveys and then we will stay here and do plankton samples...

LN: They're comparing the corals here with the corals in two other nearby sites right here in the Gulf of Chiriquí.

LN: What are the names of the other sites?

SC: Uvas is that way and Bahia Damas is the one that's along the coast of the main island.

LN: And they have three more sites in Las Perlas archipelago, about 200 miles east of Coiba, in the Gulf of Panama. But why focus on the Tropical Eastern Pacific... which spans parts of Costa Rica, Panama, Colombia and Ecuador? This is Sean.

SC: So we thought of the Tropical Eastern Pacific as a kind of natural laboratory for understanding how how corals cope with change because it is a difficult environment to begin with and because within this region there are areas that are subject to more fluctuations and environmental conditions particularly fluctuations in temperature than others.

LN: As he said, certain areas in the Tropical Eastern Pacific experience seasonal upwelling and others don't. Upwelling is a phenomenon that occurs during the summer months, when a combination of ocean currents and winds changes conditions, moving nutrient-rich and cold water from the bottom of the ocean to the surface. In the Panamanian Pacific, for example, the Gulf of Chiriqui, where we are, does not experience upwelling, whereas the Las Perlas archipelago in the Gulf of Panama does experience colder temperatures and a boom in nutrients for a few months of the year. This explains why many scientists, including Laura, call it a natural laboratory.

LM: In one gulf you have upwelling happening and the other gulf you don't have upwelling happening and the currents of upwelling implies more nutrients, colder water, less oxygen, like many different environmental conditions that these corals experience. So it's it's practically like taking corals like when we take corals to the lab. What do you do? Okay. I want to do an experiment testing different nutrient concentrations. I want to run an experiment testing different temperatures. So you play with the

environmental variables in a controlled way, so you get a group of corals you put in a tank with these conditions, then you put in another tank other conditions and then you compare with control. So what we have in Panama is like you have a for example the region that is not there's no upwelling happening it's like a control. Okay, and then the other one the other population is facing same species but facing different conditions. It is like applying a treatment in these corals, but it's in nature and it's amazing because it's much more realistic. It's it's much more complex. So that's why it's so special here in Panama. You have the same species. So, it's super comparable the different places and you have like a more stable place and a place with several environmental variables acting so it's like a natural laboratory. It's a perfect spot to understand more about how... how environmental variables are acting on these species.

LN: Although the ROHR reef resilience project looks at the entire ecosystem, Laura's research hypothesis is directly related to corals.

LM: And we believe the hypothesis is because of this variation corals from Las Perlas, for example, go through they become more robust because they're obliged to face these oscillations and cope with it somehow. So we believe that they may present some traits because of this natural variation that they go through their whole lives. If they are challenged with like, for example climate change, they can be more resistant and resilient because they are like bolder a little bit, so...

LN: And the way she's doing this is by collecting fragments from the different coral communities in the upwelling and non-upwelling sites and stressing them out in the laboratory to see how they respond, particularly regarding their physiology, which is Laura's expertise.

LM: Coral physiology is like well think about human physiology. We understand when you read about human physiology like how your body function right? That's what I do with corals. Let's say. Basically, that's it. How the body, how we responds to...to things in the environment. It's all about that how we function inside. Which is super interesting because we share so much with them...

LN: Yes, you heard it right. We, as humans, share a lot with corals... at least when it comes to our cellular biology. Some of you may not know that corals are animals. And that what we know as coral reefs are actually thousands of tiny coral polyps –each one a tiny animal- that secrete layers of calcium carbonate. These calcium carbonate secretions form the hard reef skeleton where the coral polyps live. So how did Laura become interested in corals in the first place?

LM: Okay, so I come from a countryside actually in Brazil. I'm from Sao Paulo, but from the countryside, so it's at least four or five hours to get to the closest beach. But luckily, when I was young my parents would take me to the beach and the memory I have... this is very vague, but that's quite a strong memory I have like always looking to the ocean like huge and like wondering what is... what is happening there? Like very little like this like water like curious? And I think that was my first connection. Let's say.

LN: When she grew up, she began studying marine biology, but without a clear idea of her focus... until she saw a video of corals reproducing.

LM: But then the first time I saw a coral, a video of a coral reproducing and that I become aware with the whole synchronization with the moon phase. There was like super romantic for me. Like that's it. Like I fell in love. It's a bit irrational. Okay, there's not like a rational explanation. I got so fascinated by the subject that I started to read more and more about coral reefs, corals the way of reproduction, physiology, got interested in how pollution and other aspects in the world could affect them...

LN: She was officially obsessed with corals, and eventually became passionate about their physiology... but, according to Laura, coral physiology was not a very common research field in Brazil. So when she began to search for potential mentors, there weren't that many options. She eventually found an animal physiologist who was up to the challenge, and became her advisor, but the logistics weren't ideal either...

LM: But then to work on coral reefs, I would have to travel like 3,000 kilometers to go to the field do experiments or things like this monitoring and stuff and then bring the corals down south to actually have a good environment to to work in the laboratory and like facilities.

LN: After her PhD, Laura began working in a lab in Monaco, where she finally wasn't the only coral physiologist around. But she was mainly conducting lab work and missed being in the ocean. That's how she ended up in Panama as a postdoctoral fellow in the ROHR reef resilience project. Here she has a balance between the lab work and field work. So, now that she's here, how does Laura stress out these tiny corals in the lab?

LM: We make them go through challenges like increasing temperatures to see how they respond, decreasing temperatures and many ways that we can manipulate environmental variables. So in 2020 we collect the six sites and we we ran six like six identical experiments to see their tolerance.

LN: And by subjecting them to stressors such as extreme temperature changes in the lab, she can achieve faster results than if she waited for environmental changes to eventually occur in the coral's natural habitat, such as warmer oceans due to the appearance of the El Niño phenomenon, which could take years.

LM: But that was our way to assess in a shorter period of time if okay, does these guys coming from different sites are they gonna respond differently based on the fact that they're facing upwelling or not?

LN: And these experiments don't need much coral, just some small fragments. Removing them doesn't cause damage to the reef.

LM: And many of the the analyses are super sensitive. So, we don't really need a lot of material. So just taking some tips and not damaging the colony we have enough to analyze, and we are sure that this colony is going to be able to you know, it's not gonna die. And we know that they're gonna be healthy and that we're not damaging it in a, like strong way. So, then we can follow them for years, for four years for example, and they're just doing fine.

LN: When Laura collects these small coral samples, she's taking fragments of coral skeleton with the polyps that live within them. And this polyp tissue is what detaches from the coral skeleton with the water pressure from the airbrush. But during her dives to collect these samples, she's not only thinking about her experiments. She's also gathering coral pieces for many other labs that are collaborating in the ROHR reef resilience project. This is Helio Quintero, a Panamanian marine biologist and fellow at STRI who works on the project. During the trip to Coiba, he was helping separate the coral samples that would go on to the different experiments.

Helio Quintero: Entonces, por ejemplo, aquí este tubo grande de 50 mililitros va para morfología, análisis morfológico...

LN: A piece of coral was collected for morphological analyses, to understand its shape and the differences among coral variants in the reef.

HQ: Y el primero que colocamos va en metanol, que es para estudiar metabolómica...

LN: The first sample collected went in methanol for metabolomics analyses, which is sort of like coral metabolism.

HQ: Y este que va acá va como en un líquido especial que se llama DNA/RNA shield que es como un jabón que protege el ADN super bien y va para análisis genómicos de alta calidad.

LN: And this one goes in a special liquid called DNA/RNA shield that protects the DNA for later, when they read its genetic code.

HQ: Y lo que queda acá entonces son usados para isótopos y análisis bioquímicos...

LN: And then some go to isotopic and biochemical analyses...

HQ: Aquí entonces van los pedacitos de Laura que son chiquitos pero completos...

LN: Laura's small pieces went in liquid nitrogen, a fluid that maintains the samples at –320°F, keeping them stable until they made it back to the lab.

HQ: El nitrógeno líquido básicamente pausa todo lo que está pasando en el coral y queda congelado en el tiempo listo para el análisis...

LN: The liquid nitrogen basically pauses everything that is happening in the coral, so that it stays frozen in time until it is time for the analysis. But let's fast forward and return to the lab with Laura.

LM: Because when we collect them and we freeze them it's like snapshot like you know. Stop here, and I want to see the scenario what's happening the cellular level and I can access it in. It depends on what you want to evaluate the immune system...

So Nunu is going to show you first how we extract the tissues, for then afterwards we come and measure the proteins. 03:45 So we're going to do this with those corals you saw us collecting in the reef.

LN: Nunu, short for Eunice Tapia, is a biology student from the University of Panama and an intern in Laura's lab. She showed me how the coral tissue was removed from its hard skeleton.

Eunice Tapia: Lo primero que voy a hacer es buscar el coral a –80. Se mantiene así en aluminio porque una de las pruebas que vamos a hacer es la cantidad de clorofila que tiene el coral.

LN: The first thing I'm going to do is take the coral that is stored at –80 degrees Celsius. It is stored in aluminum foil because one of the tests that we are going to run is the amount of chlorophyll in the coral.

ET: Laura fue a buscar agua de mar, ella la vamos a utilizar para que salga a presión y así sacarle el tejido.

LN: Laura went to get sea water, which we will use under pressure to extract the tissue. After the tissue extraction, the resulting liquid was homogenized. And separated into three samples: protein, chlorophyll and zooxanthellae. The amount of protein, as a measure of coral biomass and health.

LM: It is an indicator of biomass... because we have lipids, we have proteins, this is a very common indicator of biomass of the body.

LN: So higher biomass is always better health?

LM: Yeah, its kind of, like that's how we use it here. Because also producing protein, maintaining, that all takes energy. So if you're in a good state, better, if you start to decline with it that means like probably, you're spending, or you don't have energy to produce them or maybe you're using proteins because you're being stressed...

LN: And remember how we said that corals and humans have a lot in common? Well, as it turns out, this protein test can be used to evaluate human health too. In addition to the protein analyses, Laura performs several other tests:

LM: The chlorophyll, which is the pigment in the algae, and the number of algae inside the tissue.

LN: The algae inside the coral tissue, also known as zooxanthellae, live in symbiosis with corals and help them survive. Chlorophyll, in turn, helps the algae absorb energy from the sun. Part of this energy goes to the corals as well.

LM: And then we do run several others, but it's more related to the biochemical mechanisms inside the coral.

LN: One of these other tests is, for example, what leads the coral to expel its zooxanthellae or algae, which could lead to the coral's death. This process is known as coral bleaching, because without its algae, the coral reefs turn white.

LM: For the biochemistry, I'll usually work on aspects of oxidative stress which is related to redox balance. It's connected, how can I say, to free radicals for humans. So we show the same mechanisms as human... corals show the same mechanisms as humans for this redox balance, and this is completely connected to the process of bleaching in corals. So I measure total antioxidant capacity, the oxidative damage to lipids, to proteins, to DNA...

LN: Bottom line, she's hoping to see if corals from areas with upwelling, like the bay of Panama, are more resilient than those from areas without upwelling in the Panamanian Pacific, like the gulf of Chiriquí, and if so, what physiological aspects of the coral could explain this resilience.

LM: Visually, we can see the bleach, but this is usually a very severe state. Through these analyses we can see much before if they're already bleaching or not that in visually we wouldn't be able to distinguish.

LN: When diving you can tell if the coral has bleached because it is all white, but Laura hopes to detect what leads to the bleaching much earlier in the process. Think of it as the screening tests run on humans for the early detection of certain diseases. That day in the lab, she showed me how she measured the amount of protein from the tissues extracted with the airbrush.

LM: So because this reagent is considered toxic, we do only on the hood. Okay, so I just put a little bit in here...

LN: The hood is a piece of equipment common in labs that captures and removes toxic vapors, gases and other elements. So, Laura was sitting behind a safety glass while mixing her coral tissue samples with the toxic reagent: a copper solution.

LM: This is a very concentrated solution of copper. Actually that's allows, it binds with the the proteins in the sample. I don't know how to explain really in detail. But it's that's why it's such a blue solution because it's highly copper high in a very high concentration.

LN: When mixed with the coral tissue samples, the copper binds to the protein present. This allows Laura to run it through a machine that tells her how much protein was in that coral. That day, she was running seventeen samples. She had 30 minutes to get it done after mixing in the copper before the samples destabilized. But when we got back to Laura's lab, the equipment didn't seem to be working.

LM: Guys are we having energy problems here? Because I can't turn it on anything...

LN: The whole side of the lab where analyses were run was suddenly out of power. And Laura began to worry. All that effort: scuba diving to collect samples from 17 different corals in the field, freezing them until their tissues were extracted, crushed and combined with a copper solution, were at risk of being lost forever.

LM: This is connected here...but you see it doesn't turn it on. Oh no no... Yeah, I have to read I'm gonna read not to lose the samples. Luckily, this is like stable for half an hour, so I don't want to lose my samples...

LN: Fortunately, the problem was solved within 10 minutes, and she was able to process her samples.

LM: It's reading and in like five seconds, you're gonna see a bunch of numbers popping up on the screen and through these numbers then we calculate and we run analysis statistical analysis things like this. So all the numbers, all the numbers... and the more... the darker it is, the more concentrated it is. That's the logic.

LN: So, what happens after we understand which corals have more protein content, more chlorophyll or more algae? This information can be contrasted with the results of the experiments done on corals from the bay of Panama and the gulf of Chiriqui subjected to extreme temperatures in the lab. In turn, providing clues about whether corals facing upwelling conditions could be more resilient and also what specific aspects of their physiology could be

building their immunity against climate change. And this is important because... well, I'll let Laura explain it:

LM: This is extremely important because in the end it's the combination of stressors that matters. It's never just temperature. It's not just pollution. It's not just the plastic. It's not like everything is acting together. Because also for us to develop, to develop like techniques or solutions to mitigate some things, you have to understand what's happening there. How am I going to come up with a solution if I don't even know really what's happening in the place?

LN: And the time to understand it is now, that's why the ROHR reef resilience project is so important and timely. This is Sean.

SC: We have a limited amount of time to understand how this... How this ecosystem works when it's not in a severely degraded state. So, this is kind of a relatively narrow window to learn as much as we can about a system before it's it's transformed in some quite dramatic ways. We have to know what the ecosystem was like and how it worked in order to think about restoring right? And so having that sort of basic understanding of how these systems operate understanding how species interact is critical knowledge if you... if you want to understand how successful you are at rebuilding reef communities later on.

LN: This research receives funding from the Mark and Rachel Rohr Foundation. Our podcast team includes me, Leila Nilipour, as well as Ana Endara, Linette Dutari, Elisabeth King, Lina González, Johann González, Jess Sadeq and Sharon Bryant. Episode artwork is by Paulette Guardia. Extra support comes from PRX. Our show is mixed by Melissa Pinel. Our episode's music is by Epidemic Sound. If you liked this episode, please share it with other people. And thank you for listening.