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I: Welcome to IFLScience The Big Questions. I am Dr Alfredo Carpineti, Senior Science Writer and Space Correspondent and I'll be your host in this episode. Drug discoveries are fundamental to treat old and new diseases. A lot of the work done takes inspiration from nature, looking at compounds already being produced by living creatures. A relatively untapped source of exploration is the deep ocean and its inhabitants. For this episode we are joined by Dr Sam Afoullouss from the University of South Florida to take a figurative dive right into the subject.

Thank you Sam for joining us.

R: Thank you for having me.

I: Would you like to tell us a little bit more about yourself and your work?

R: Yep. So my name is Sam Afoullouss. I'm a post doc in the University of South Florida working in the Baker lab under Dr Bill Baker who is one of the pinnacles of this field, marine natural products, and my job at the moment is currently exploring deep sea sponges and corals to see what kind of potential medicines they might have and how we can integrate that data into a larger understanding of deep sea ecosystems, drug discovery, and then some kind of chemical ecology. So, how animals talk to each other using chemicals, how they defend themselves and how those systems kinda work.

I: You work sounds absolutely incredible and we're curious to know what sparked your interest in exploring the deep sea for medical compounds.

R: That was actually something, it was a happy confluence. I studied for my degree in the University of Galway where I was studying biopharmaceutical chemistry which is just a fancy way of saying chemistry with the aim of drug development, and while I was doing my chemistry degree I was volunteering in a zoology lab that was a pretty cool lab, it was the venom systems run by Dr Michel Dugon and this was a lab where they were looking at different venomous animals and trying to use those venoms to create medicines, like anti-cancer drugs or antibiotics and things like that. It was when I was volunteering in that lab I kinda got that concept in my head of turning to nature to try and find our medicines and at the same time, in my spare time, I joined the SCUBA diving club in the college and started to learn how to dive. Ireland is not one of those tropical places, it's pretty cold water and whenever you look at the sea you don't necessarily see it as inviting, but after doing the training and then when I got my first dive and got in the water and started seeing these amazing alien type creatures, my mind was kind of blown. In Ireland we don't have any big wild ecosystems like you do the big woodlands in Europe or the Savannah, so for us to see true wild ecosystems is pretty hard.

But once you put the scuba diving mask on and you go under the water, it's completely wild and there are forms of life that you have no idea what they are and it just sparks this curiosity. When I was finishing up my degree in chemistry I had a little bit of the taste of research from the venom lab and I loved the marine world. A position opened up on this deep-sea project that I ended up joining and doing my PhD on which was a match made in heaven, right? My personal passion of the marine biology and the marine world combined with my professional passion for chemistry and drug discovery and it was a really nice confluence. As a SCUBA diver one of the things that always kind of entices a lot of SCUBA divers is the ability to go deeper and deeper and to see what's there. There is only so deep you can go SCUBA diving before you start to deal with dangers from the high pressure of the weight of the water pressing down on you, how much gas you will need to survive. I was always limited to the 30 metre, 40 metre mark and then when I was introduced to deep sea projects with an ROV or robotic submarine the size of a minibus that can go down to 3 kilometres, that immediately just lit up my brain as "oh what's down there?" you know? Because what I would have noticed as a SCUBA diver is the most life is in maybe the top three or four metres and then as you go down the abundance decreases but then when you get down to the deep sea the abundance then rises up again. It was something that I was really curious to explore, so it was a nice natural process to get into it.

I: Thank you for that. You already gave me a couple of challenges; one is specific to Ireland being very cold in the water and the other one is just that human research underwater is limited to obviously the pressure that a scuba diver can take. What kind of challenges in general there are in conducting research in the deep-sea environment and how do we overcome them?

R: Until recently, it's been something that's incredibly difficult to do, right? Obviously it's too deep to dive down because of the hydrostatic pressures, the force of the water around you, and then even to get equipment that can go down to that depth, scientific equipment which tends to be very sensitive, it is quite a challenge. There was a bit of a flurry of deep-sea exploration in the 1970's with submersibles that were developed for commercial use, oil rigs and that kind of work and scientists managed to persuade some, presumably, fairly wealthy people in institutes to let them have time on these instruments to start to explore. Then there was a little bit of a die off when they realised manned submersibles are really expensive to run. There's a high danger risk with them and the utility of them isn't as great as what would come later which was the ROV's or the remotely operated vehicles. Because it's an extreme environment, putting people in there adds danger which then increased cost and risk, whereas when you have a remotely operated vehicle with state-of-the-art robotics it allows you to not only go down to those depths to analyse them with 4k video footage with big floodlights, it's a pitch-black environment. There's a complete absence of all light so you need to bring down powerful video lights and floodlights to be able to see what you're sampling but then also advances in robotics in terms of the manipulator arms. So, to collect just, say, a specimen, you need to have a really malleable and delicate collector arm and that only became… those developments only started from the 90's onwards. Even the cable that connects the ROV, so this remotely operated vehicle to the surface ship. So the surface ship might be at the top, so the ROV might be three kilometres below, so that might mean the cable is five kilometres and if you've ever tried to use a mouse on your computer when there is lag, you know how difficult that can be.

So, because these cables are so long they have to use fibre optics to prevent there being any lag because it would make it impossible to collect specimens. It's not only the physical ability to collect the samples but also knowing where to find them. In the early 1800's people started to map out the deep sea and tried with drop lines, they can measure certain sections, but we never really had high resolution maps of the deep sea and especially the most important region of the deep sea, in terms of my work and in terms of biodiversity so what we call the continental shelf. That's where the sea floor drops from 300 metres down to 3,000 metres plateauing out at around 3,500 to 4,000 metres. That happens over a very short period of time and its kind of the equivalent of a giant cliff along the continental shelf and it's hidden within the little nooks and crannies, the equivalent of fjords within this geographical landscape that the coral reefs are hidden. It was only in the early 2000's that we got, in Europe, in Ireland anyway, we got the sonars required to do high resolution mapping of the sea floor which is vital for finding those little nooks and crannies, because without that you're kind of searching in the dark. So it was a combination of a state-of-the-art robot that could spend… I think some of our dives were 16 hours, 17 hours, on the sea floor that needs to have a very sensitive manipulator arm for sustainably collecting samples, and then you also need to know where to look, so the sea floor mapping. I think the stats still stands correct. We've higher resolution maps of the moon than we do of our sea floor which is something that's pretty astounding. That plays a huge part and in the past ten years there's been a huge development in autonomous underwater vehicles. So basically you launch this torpedo looking device from one of the research vessels, you tell it to map what you want it to scan and it will dive down to the deep sea and do high resolution sea floor mapping and then pop back up to the surface, send off a satellite signal to let you know it's finished mapping and then a boat goes out and collects it. So, that allows a research crew to scan five or six times more sea floor to get these high-resolution maps in one trip versus just bringing the multi-beam sonar behind the boat. So there has been a few advances in the mapping and the actual process of collecting the samples that have been pretty vital for our work.

I: I love it. I love how humans can get really clever, how we do science when we want to. So I think it's time that we move on to the chemistry and talk about one of the intriguing compounds that you have been studying. It's called Ziconotide.

R: Yep Ziconotide.

I: And it's a painkiller that has been extracted from a sea snail. Can you tell us a little bit more about this compound and what makes it unique?

R: Yeah. So this was a really interesting example of a marine natural product. I think it was in the early 2000's, it was isolated from a sea snail called the *Conus Magnus* or the Magical Cone Snail and what this compound is, is an incredibly potent neurotoxin. So it paralyses the nervous system instantly and in terms of why would a sea snail produce something like this, a neurotoxin that's a thousand times stronger than Morphine? Well it turns out its down to its biology and how it hunts. So, these sea snails, they don't have the speed to chase down their prey, they're snails and they eat fish. So what they've developed is this small harpoon that once the fish swim past them, they shoot out and inject the fish with this potent neurotoxin which paralyses it instantly, meaning that the fish doesn't have the chance to swim away from the snail and if the fish does swim away, it won't swim too far that the snail won't be able to catch it before some other predator does. When researchers started studying this and started looking at the venom and realised that, hang on, this is a really potent pain killer or really potent neurotoxin, they figured out that hang on, this might be a useful pain killer, if we can turn off the nerves in an area that's sore it will be an effective painkiller. So, they went through a few different phases and they managed to figure out a way of manufacturing it in a sustainable manner without having to go back to the sea and collect all these sea snails and it's used now as one of the strongest painkillers you can get. It's a thousand times stronger than morphine which kind of blows my mind a bit. One of the big benefits of it is unlike opioids that are traditionally used for painkillers, whether it's morphine, fentanyl is used a lot in hospitals, it doesn't have the addictive properties of the opioids because it's not an opioid based drug. So there is a huge benefit to that. We know in the US, for example, there is huge issues with people getting addictions to opioids post-surgery and things like that, that will lead to substance abuse issues so thankfully for this compound, Ziconotide, I think the drug name is Prialt, it doesn't have those addictive side effects. So, it shows how although Morphine was found from nature, it's an example of a natural product, there are other compounds in nature that we can use for our medicinal needs that have some added benefits that we need to try to integrate.

I: Thank you so much, it's absolutely brilliant. I found it so, so incredible that we can get to this kind of product. Can you tell us, how do you envision for the future of medicine extracted from the deep sea? Are there a specific area or specific species that look promising or is it just like we could find anything anywhere, we just need to look for it?

R: It's a little bit of both. Because the deep sea is so underexplored, there is a little bit of no matter where we look we will find something new. I think it's less than 0.1 percent of the deep sea has been sampled, so specimens, whether it's sediment or coral sponges, that type of thing, have actually been sampled and brought up. So it's almost no matter where we look, I think we're going to find some new things. The areas that I think are of the most interest and the most exciting are complex biological ecosystems that have a lot of competition. So that's going to be things like different types of reefs, we have the cold-water coral reefs where the corals and sponges are what we call ecosystem engineers. So they'll grow on rocky outcrops where there is a strong flow of water and as they build their three-dimensional structure, sponges and corals are the marine equivalent almost of plants or trees, right? The same way that a tree builds a structure, builds a forest and the bird life and the hedgehogs and everything can come in, it's the same story with the corals and sponges. They build these complex ecosystems with little nooks and crannies that other animals can come in and inhabit, like sea stars, like arthropods, like little fish will use them as hatcheries or octopus and when they create these ecosystems it will then result in some form of competition. Whether it's competition between the sponges and corals fighting for space to feed in the water current, whether it's prey/predator type competition. Maybe the starfish are interested in eating the sponges or the corals so then they will produce compounds to defend themselves from predators, anti-feeding type behaviour. There's also some really cool stuff that's not necessarily drug related but still really really exciting. A lot of the corals we collected in the deep-sea bioluminesce when you touch them, so they glow in the dark and you're thinking, they're in an ecosystem or an environment where there's no light, like absolute pitch blackness, but they produce light. So, why they think they're doing this is if you touch the coral and it produces light, well if a predator comes along and tried to eat the coral polyps, he's going to be nibbling on the coral and causing it to produce light which might attract a fish that might eat the… if it's a starfish say, or a polychaete, it will put out a warning siren and other fish will see that and come in and eat the predator. There are these cool chemical interactions between organisms that I think that's where the future is and it's not only on the macro-scale in terms of sponges and corals fighting with each other for space but also on the micro-scale in terms of the microbiome of these organisms. So, just say for example, sponges, the vast majority of the weight of most sponges is bacteria that's living within them, bacteria and fungus living within them, and a lot of the chemistry that they produce, and sponges and corals have been shown to produce really interesting what we call secondary metabolites or specialised metabolites, these are the ones that tend to be unique to an individual organism, a lot of the cool compounds that they produce turn out to be from their microbiome in symbiotic mutualistic relationships and I think that's definitely a future element of it. A lot of the compounds that we'll find, they have what are called chiral centres, so basically they can have a lot of mirror images of each other and that massively affects how their biological activities will work. Like thalidomide is a great example, the left-hand version of the molecule was a great morning sickness drug whereas the right-hand version of the molecule caused birth defects and mutations, so the same 2D structure or the same shape drawn out on a page but one was the left-hand version, one was the right-hand version. So chirality is something that's important when it comes to drug discovery and a lot of the compounds we find have lots of these chiral centres which make them really hard to manufacture in a lab but because a lot of these compounds are produced by the microbes that live within the sponges or the coral, or the sponges and coral have DNA which encodes for these interesting cool compounds, I think that's going to be the future of drug discovery where we look at the DNA of these animals and see, okay, this is the gene for producing this anti-malarial drug, why don't we take that gene from the coral and put it, just say, into yeast or into *E. coli* and grow them up in a bioreactor? They will then make that potential drug, the future medicine that without needing… so you can produce it on a sustainable scale just with bioreactors, something we have been doing for thousands of years making beer, right, same kind of concept just instead of the bioreactor creating beer, we are creating our next generation of anti-malarial compounds or anti-cancer compounds and you can do it in a much more sustainable way because you don't need a lot of the harsh chemicals that are used to synthesise molecules, like heavy metal catalysts or some kind of extreme solvents because you can do it in a biological process it can be a lot more cost effective, time effective and environmentally friendly. So, I really think the future is going to be using the biological recipes within the DNA to then produce the molecules rather than having to go back to the deep sea to harvest or to try and synthesise it in a lab, which a lot of the times you can find a bioactive compound that would make a fantastic drug but because it's too complex to make and it couldn't be done cost effectively or efficiently, it doesn't go onto further stages of clinical trials and end up on the market. So, having a sustainable source of these compounds is really important and supports the initial level of work we do because we could always look at the DNA and use that to make the molecule rather than having to make it in the lab.

I: Thank you for that. Also because you already started answering my next question. You mentioned that when we were talking about the sea snail that you don't want to just go back and harvest as many sea snails as possible just to get the compound, was to understand the compound. Now you discuss how it's just copying the instruction already in nature and using bioreactors to produce these compounds. So, it seems to me that actually this research for new medical compounds aligns very well with the preservation of deep-sea ecosystem.

R: One of the lessons I learned going out to sea was how important the sustainability element of my work is and how important it is to be sustainable when you're collecting these specimens. Some of these corals take thousands of years to grow I would imagine. There were bamboo corals that the trunk of the bamboo coral was as thick as a tree and they're growing in a cold environment, so they're growing slow, there's a low amount of nutrients in the deep sea which is another factor forcing them to grow slow. So these are very susceptible ecosystems because they grow so slowly. There was one area I remember particularly well, two or three dives before that where we dropped the ROV in, it takes nearly an hour for the ROV to reach the sea floor, that's how long of a drop it is. Once we got down we would fly the ROV around and we saw nothing, there was maybe three dives in a row that was like that. So you're just staring at a blank screen that looks as if there's snow filtering on, then on the fourth dive we did, we were getting a little bit fed up at this stage, we dropped the ROV in again and we came across a completely pristine region of coral. It was spectacular. The colours, in deep sea there is no light, so you don't expect these organisms to be beautiful colours but beautiful pinks and purples and yellows. It was amazing and then we came across this giant sponge. It's called a Gramophone sponge, it's one of my favourite sponges I've ever seen. It's probably two metres wide, three metres tall and then maybe three or four metres deep and it bellows out from the cliff face like a gramophone. So we were just in awe, everyone was in awe, the ROV pilots were in awe and we were all just like, woah this is cool. We got the pilot to go in closer to take some nice close photos of it, get some good pictures and videos of it and as we got closer we realised there were these tiny yellow dots growing on it. These yellow dots were a coral-like animal called a Zoanthid and they're known for being rich in chemistry. So for me, this was after a day of collecting nothing, coming across this big giant sample that's also covered in Zoanthids, a known source of cool chemistry, I was ecstatic. I was like, we have to get this, we have to get this. The biologists on the boat, because the vast majority of our collection team were biologists, they were like, there's not a chance we're touching this and we started to have our debate. Is it worthwhile, you know, this organism, this sample will almost definitely have interesting compounds in it that could potentially be turned into a drug. But then on the other side, this is such a pristine area of reef, after we just did three or four dives over areas that were completely dead, how could we destroy this nice, protected bit even if our end goal is a good end goal. When there is an area that's well sheltered, well protected and undisturbed, we should really try not to disturb it, even if there is a good intention at heart. As this was the first year of my PhD I was pretty young, naïve and excited I suppose about it, and eventually it was getting pretty heated so my PI, the leader of the scientific cruise, Professor Louise Alcock, at the end of the day it was her decision so she was like, look because this area is so pristine, we're not going to collect it. We'll keep an eye out for this, we know this organism lives down here now so we're going to try and collect it later on but when you find an area that's this pristine, this untouched, this well preserved and this, frankly, beautiful, protection of the ecosystem has to be our highest priority.

I: **That is wonderful, thank you so much for sharing those stories, your first encounter with Gramophone Sponge and thank you so much for taking us on this journey to the deep sea to learn how there is so much out there that we don't know and there is such huge potential to discover interesting chemicals that might revolutionise medicine and beyond. So, thank you.**

R: No problem at all, thank you for having me.

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