Genome Insider S4 Episode 4: Methane Makers in Yosemite's Lakes - Mike Beman and Elisabet Perez Coronel

Menaka: To get where we're going today, picture a map of the state of California, and then throw a dart right in the center of it. We're going to the University of California, Merced. And from there we'll head up to the Sierra Nevada Mountains. So grab your sunscreen and your bug spray. This episode begins with the drive to Yosemite National Park.

Mike Beman: And you hop out of the car and it's over this steep, sort of trail and, you know, the first few steps, all of a sudden you realize, wow, we are up at high elevation.

Menaka: We're talking 9,000 feet above sea level,

Mike Beman: It's really hard to catch your breath.

Menaka: Partly, that's altitude, but also, these are pretty breathtaking surroundings. Across the road, a green meadow stretches out, lined with pines. Above those trees, Yosemite's granite peaks reach up into the sky.

Yosemite has many freshwater lakes nestled between meadows and granite peaks. (Elisabet Perez Coronel)

Elisabet Perez Coronel: I think it's Mount Morrison on the horizon, and it's just, the sun is starting to come out because we have to be there early.

Menaka: And like most sampling trips, all the gear we need is coming in with us. But our ride stops here, at the trailhead. We're hiking the rest of the way in, with everything we need on our backs.

Elisabet Perez Coronel: This is probably where I would warn, I would warn you that it's gonna be a little uphill. It's gonna be like half an hour uphill, like, like really uphill.

Menaka: It's hard work!

Mike Beman: But it's amazing, because then right away you get up and you have these beautiful vistas,

Elisabet Perez Coronel: So here we take a little break just because of the view

Menaka: From up here, you can see even farther into the distance. So there are peaks speckled with trees, and taller summits that are still partly covered with snow. If you look down a bit, you can also see where we're headed. It's a lake, set against a meadow, with rounded granite rocks at its shores.

Mike Beman: And then you hike down to the lake.

And that lake is where we're collecting our samples. It's a bright blue that mirrors the sky. It's quiet. It feels isolated, like this might be the only lake around.

Mike Beman: But then once you get up above to the higher elevations, you find these lakes just tucked in all over the landscape.

Menaka: And within these serene lakes, there are millions of microbes cycling carbon and other nutrients. These lakes are windows into how freshwater ecosystems fit into climate cycles. And sometimes that view is surprising. So in this episode — the researchers who took a look at these lakes. They found something they didn't expect: microbes making methane.

Menaka: This is Genome Insider from the US Department of Energy Joint Genome Institute. Where researchers discover the expertise encoded in our environment — in the genomes of plants, fungi, bacteria, archaea, algae and environmental viruses — to power a more sustainable future. I'm Menaka Wilhelm.

And in this episode you're hearing from Mike Beman, from UC Merced, and Elizabet Perez Coronel, who recently finished her PhD with Mike. They spent the last few years looking at Yosemite's lakes, and working to understand the microbes living there. So we'll get into how they collect samples — from both the middle of these lakes as well as the shore— and what they've learned.

But first, I want to take us back in time a few years, to when Mike started his work at UC Merced. Because he didn't really expect to work on lakes.

Mike started out in science looking at ocean ecosystems. In salt water, he looked at how microbes cycle nutrients, like nitrogen. So he planned to keep working on microbes, and he figured he'd stick to the ocean.

Mike Beman: And it was really just a single conversation with a professor at UCSB, John Melack, and he said, you know, you're gonna be at UC Merced, you gotta go work up in the Sierra Nevada and the lakes up there.

Menaka: Of all the University of California campuses, UC Merced is the closest to the Sierra Nevada mountain range, where Yosemite sits. But most people think of these mountains as a place to hike or rock climb.

Menaka: What stood out about the lakes in Yosemite in that conversation?

Mike Beman: Yeah, you do have these, you know, kind of these lake chains that will start all the way up, in some cases, glaciers feeding into a series of lakes that are connected by a stream. And then eventually they all connect into these large rivers that, you know, go down and flow down the mountains and out through the Central Valley and out, to the ocean. So there's some interesting things you can do there.

Menaka: For example, as lakes vary north to south, their character shifts. You see different plants, different microclimates, different nutrients and different organisms in the lakes. You can also track changes across all kinds of elevations.

Mike Beman: What you get with elevation of course is a huge change in temperature.

Menaka: Another dimension to look at. The same way Yosemite’s granite faces are like a jungle gym for climbers, these lakes were looking like a playground for a microbial ecologist. Beyond that, it’s the kind of environment where more information could help protect a special ecosystem.

Mike Beman: We see Yosemite and it looks really pristine, right? But there are a lot of different threats that the ecosystems up there face, climate change being one, there's been the spread of infectious disease for amphibians. And so they're sort of at this key point where they are facing some of these threats, but are also really pristine, beautiful environments. So they're really interesting scientifically. And then of course, just being there is, is an amazing, amazing thing.

Menaka: So that very first year he started his lab, Mike headed out to Yosemite, to collect samples. He’s kept going since. And then of course he’s brought students along, which is how he did the project we’re talking about today.

Mike Beman: You know for example, the graduate student that really pushed the project, she grew up in Mexico City, right? So it's very, although that's in the mountains right, it's a very different setting. She kind of immediately really fell in love with Yosemite.

Menaka: That graduate student was Elisabet Perez Coronel – and she did confirm that working in Yosemite was a pretty great time.

Elisabet Perez Coronel: I got to just be in this amazing environment, spent like three summers there, so it was fun. We had like a bunch of adventures too, like whenever we weren’t sampling, we could just go hiking in the areas, there's like a bunch of lakes, explore. It was really fun.

Menaka: Like Mike, when Elisabet came to Yosemite, she was curious about how microbes impact their environment, and vice versa.

Elisabet Perez Coronel: I always find this relationship between microbes, climate change, humans — very fascinating.

Menaka: And all of that brings them to this project where they eventually find new sources of methane gas in these freshwater lakes. Just to be clear, this is the same methane gas that lots of human industries release into the atmosphere. Mostly, we hear about it because we use it.

Mike Beman: It's the key component of natural gas. And so we burn it in furnaces and hot water heaters and things like that.

Menaka: And when we release methane into the atmosphere, it traps heat, and drives climate change. It's an important greenhouse gas, like carbon dioxide.

Elisabet Perez Coronel: it's not as abundant, so I guess people might think it's not as important, but in terms of comparing it with CO_2, it can even be more significant to the warming, it's just, like, less abundant.

Menaka: So understanding where methane comes from is important for building climate models, and understanding our atmosphere. And methane gets made, naturally, lots of places on Earth.

Mike Beman: soils that are waterlogged, aquatic ecosystems, of course, even, the guts of animals, especially ruminants.

Menaka: All of those environments have something in common: they're pretty sealed off from air.

Mike Beman: But what that means is that it typically can only be produced under anoxic conditions where there's no oxygen.

Menaka: And that's because a certain type of organism is often making methane in those environments. They're microbes called methanogens. They live oxygen-free lives, they're anaerobic — so they're the reason we mainly think of methane production as being something that happens without oxygen.

Elisabet Perez Coronel: Because of the physiology of methanogens, if you have oxygen present, then they would be inhibited from their function so they could not produce methane.

Menaka: So historically, here's what we've understood about methane. Microbes make it, mainly in anaerobic conditions, and it warms our atmosphere. And because understanding what's entering the atmosphere is crucial for modeling what will happen with our climate, people have been paying attention to where methane comes from for a long time. But mainly, they've looked at methane in oxygen-free environments.

Here, Mike and Elisabet are doing something different – they're looking at methane production in freshwater lakes. And that's an environment that's got plenty of oxygen. So people haven't paid too much attention to methane coming from places like this.

Mike Beman: But if you have plants and freshwater organisms and marine organisms that are producing it, then that makes the models a lot more complicated.

Because if methane could be coming from an oxygenated lake, it could be coming from other environments we didn't expect before, too.

And there's actually another layer here, to why you wouldn't really expect methane in a lake. According to the dogma of methanogens-make-methane-without-oxygen, there's really only one place that methane-makers could live in a lake: at the very bottom, in the mud, where the oxygen dissolved in the lake water couldn't reach them.

And then — there are other organisms in a lake that break down methane. They're known to live throughout the water column, and they're very good at that job. So you'd expect that those microbes would break down whatever methane made its way up from the bottom of a lake. None of that methane gas would reach the upper waters of the lake, and certainly not the atmosphere. But there turns out to be a bit of a hole in that theory.

Elisabet Perez Coronel: What is being recorded for, like, decades at this point, like if you really go back into the literature, people would be saying, "Oh, we saw some methane accumulation in the surface waters. That's odd."

Menaka: Because the only way for methane to exist at the top of a lake, would be for organisms to produce it up there, where they might be living an oxygenated life.

Elisabet Perez Coronel: For the most part it was like, oh, you know, interesting observation.

Menaka: Finding methane at the top of a lake wasn't really explainable. So it got its own name – the methane paradox. Because if methanogens could only make methane sealed off from oxygen, but somehow, methane appeared in this oxygenated part of the lake, the ideas hit a traffic jam and come to a stand still.

Elisabet Perez Coronel: So when I first started, like I knew about the methane paradox, I read about it and I thought it was cool, but I, we didn't know how widespread it was at the time.

Menaka: Sampling at Yosemite's mountaintop lakes would change that. So let's get back to the sampling trip we started at the beginning of this episode. These collections happen at alpine lakes – high elevation, low human disturbance, researchers carrying everything in and out in backpacks.

Mike Beman: And we collect a range of samples that we bring back out so that, that can be a challenge. And we take a few measurements in the lake,

Menaka: So they measure wind speed, temperature, and oxygen saturation in the lake. And of course, they bring some of that lake water back to the lab.

To collect samples, Elisabet Perez Coronel often rafted out into these lakes. (Elisabet Perez Coronel, Mike Beman)

Mike Beman: A lot of times what we'll do is we'll collect water samples either from an inflatable raft, that we actually inflate and go out onto the lake, or from shore, depending on what we're doing.

Menaka: When she was working on this project, the brave rafter was Elisabet.

Elisabet Perez Coronel: I was the one that would actually go in the water to collect the water samples and make the measurements, like in the lake, like in the middle of the lake.

Menaka: She told me the raft was small – not too much bigger than an inner tube, so it was easy enough to hike with it and a small paddle. From the middle of the lake, she'd measure the lake, and take samples as well.

Mike Beman: So we collect the water into these bottles that we can seal, so they're airtight.

Menaka:That's key – the goal is for samples to stay consistent from collection all the way back to the lab.

Menaka: I think most people when they think about sampling, they think about getting their samples like immediately on ice, it doesn't sound like that would be an option for you but — is it?

Mike Beman: Mike Beman: Well, so a couple of times, so it depends what we're doing, but, on those longer trips, I actually took a cooler with some dry ice in it to put our samples to, to freeze them right away.

Menaka: And dry ice turns out to be an intriguing new substance for wildlife in Yosemite.

A curious marmot inspects the samples in a cooler. (Mike Beman)

Mike Beman: And the funny thing was, there was a very curious marmot,

Menaka: So marmots are big rodents. Picture a squirrel the size of a housecat, with the teeth of a beaver and the gentle vibe of a chipmunk. They live in the mountains around the world. So you can find them in Northwest America and the Alps in Europe. And you often hear them from a distance before you see them — they've got quite an alarm call. And like Mike said, this was a marmot that seemed to be interested in the samples.

Mike Beman: That came and, kept checking out the cooler that had the samples in it. So we were worried the marmot was gonna try to steal the DNA samples, Menaka: You had a marmot interested in genomics!

Mike Beman: Yeah, we should credit the marmot as a co-author, I think, on that.

Menaka: So with the marmot's blessing, they get a bunch of water samples back to the lab at UC Merced. And they're looking at the methane concentration in those samples.

Elisabet Perez Coronel: What I was expecting to see was, like, consumption of methane.

Menaka: So basically, those lake microbes that break down methane would do their job, and chomp away at methane, lowering the concentration over time. But that's not what happened. The concentration of methane went up over time.

Elisabet Perez Coronel: So we were like, "Hmm, interesting."

Menaka: So, naturally, they took a closer look. We'll get into what they found after just a quick break.

Allison: The JGI supported this project via the Community Science Program. This program provides genomics resources for projects with Department of Energy relevance. And we accept proposals from scientists at all career stages. But you don't have to take it from us. Here's Mike:

Mike Beman:

The thing that was really useful with the CSP is that it allows you to do some sequencing, right? So that you can actually look at what's in some of these samples, what organisms, what they're capable of. And so pretty quickly after we received the CSP, we sequenced a couple of metagenomes, and that's where we started to see these particular organisms. And those were included in, in some of the subsequent analyses that assembled the MAGs that turned out to be really useful. And then we were also able to get some 16S sequences so we could look at all of our samples and experiments too and, and see how different, the abundance of different organisms was changing in those. And so that was really useful in actually excluding some of the things that we thought could be important. And then finally after we had a much better idea on what was happening, we were able to have metagenomes sequenced from some of our different experiments so that we could see the potential effects in some of those. It was great because we had that ability to get initial data, the flexibility to, to analyze things as needed, and to get the really nice, analyzed data back. So we could jump right into it right away.

Allison: You can find out more about submitting proposals to the JGI on our website. Head to joint geno.me/proposals. And hey if you want to mix and mingle with our users and stakeholders in person, consider attending our annual User Meeting in person. Visit jointgeno.me/JGI2023. We've also got a link to our website waiting for you, wherever you're listening to this episode —

either in the episode description, or the show notes.

Menaka: This is Genome Insider, from the JGI. To recap where we've been so far, we're following UC Merced scientists Mike Beman and Elisabet Perez Coronel, who've studied the lakes of Yosemite. And they've seen something a bit surprising. In lake water samples they've brought back to the lab, methane gas is increasing over time — it's getting made in that water. And that's surprising because for decades, people thought that was impossible. Here's Mike.

Mike Beman: It turns out there's a lot of methane in the, kind of, the upper surface waters of the lakes that we study, and lakes around the world and the ocean too. And in order for that to be there, it must be produced in some way. So the question has been, well, how is that taking place? because there's plenty of oxygen there. It can't be typical methane production.

Menaka: So Mike and Elisabet run through some possibilities for what could be going on. Maybe, inside little bits of decomposing leaves, or waste, there are pockets where there's no oxygen, where typical methane makers, those methanogens, could operate. Here's Elisabet with the other idea they kicked around.

Elisabet Perez Coronel: That it is not methanogens, but there's like other organisms that are producing methane.

Menaka: Maybe algae, or bacteria were the source of this methane gas. That hadn't been studied much in freshwater, but other researchers had seen bacteria make methane in the water where Mike Beman started his career — the ocean.

Mike Beman: And so people recognized in the ocean, 40 years ago,

Menaka: But in freshwater, this is a newer idea –

Mike Beman: And then, what's happened over the last, you know, 13-14 years is people keep finding these different mechanisms that could potentially produce methane in the presence of oxygen.

Menaka: So Mike and Elisabet had a couple ideas about how methane could be appearing in their oxygenated samples. But it would be a bit of a tricky question to answer.

Mike Beman: One of the challenges with this methane paradox is how do you find something, that's hard to find and maybe find something new, as well?

Menaka: It's that challenge that's common to questions like this — if you don't know what you're looking for, how do you choose the right tools for your search? So first, they looked around, to see what other researchers had found.

Mike Beman: This paper came out that showed that cyanobacteria, so freshwater, photosynthetic, cyanobacteria and marine as well, could produce methane.

Menaka: A non-methanogen making methane! Seemed like a good road to go down – so they took a look at what cyanobacteria were up to in their samples.

Mike Beman: But when we looked at that phenomenon, we didn't see a signal with cyanobacteria. In fact, they weren't that common in some of those experiments.

Menaka: Not cyanobacteria after all — but there was a thread in that work that stood out to Mike and Elisabet. The paper didn't have an explanation, but it noted an activity associated with higher methane levels.

Mike Beman: And they think it's linked to photosynthesis, based on the fact that it happens when they're exposed to light.

Menaka: And sure enough, when Mike and Elisabet exposed their samples to a treatment of light, this was true, too. Higher light levels made more methane.

Mike Beman: So we knew that there was something going on with light that was leading to methane production. But we have no idea why, you know, how or why.

Menaka: With the JGI's Community Science Program, they had a lot of different places to look for clues about the mechanisms behind this methane production.

Elisabet Perez Coronel: And in addition of doing the treatment, we had like the metagenomes, the metatranscriptomes, 16S sequencing and, and stable isotopes. to kind of try to figure out — how.

Menaka: So they'd take those lake water samples, expose them to different levels of light, then filter them for genetic material that the JGI would analyze. So that's our sending-samples-into-the JGI moment of this episode. And then, of course, data comes back.

Elisabet Perez Coronel: Fast forward, like, we sequence everything. We got like our metatranscriptomes, we got metagenomes,

Menaka: Which is exciting – but can also be its own kind of challenge.

Elisabet Perez Coronel: At that point when I was doing the data exploration, you know, like we just had so much data.

Menaka: I'm probably being dramatic, but that's a mountain of data, almost as daunting as Yosemite's El Capitan rock face. They'd need a way to climb that mountain of information, to compare organisms and pathways. And, actually, like any good belay partner, the JGI had some tips for that climbing route.

So let’s talk a little bit about the JGI’s resources for analysis. After the JGI pulls these different kinds of data out of samples, like Mike and Elisabet’s lake water, those sequences live online, in a database called Integrated Microbial Genomes and Microbiomes – IMG/M, for short.

Rekha Seshadri: And then, on top of that, you have the UI, which allows the users to sort of explore that data. Not just find it, but also compare it.

Menaka: That’s Rekha Seshadri, one of the scientists behind IMG/M. Online, at img.jgi.doe.gov, researchers can access lots of data, including their own, and they can also use software available there to look for differences and similarities within that data. IMG/M is set up very specifically, so that it’s possible to compare different datasets cleanly. Here’s Natalia Ivanova, another scientist behind IMG/M.

Natalia Ivanova: The important kind of caveat for being able to do comparative genomics is that things have to be annotated exactly the same way, because otherwise you will be comparing artifacts of annotation, of essentially data processing, not of biology that you have behind.

Menaka: IMG puts all data through the same pipeline, so that researchers can make solid comparisons.

Rekha Seshadri: You know, and we like to think of the data sort of serving all kinds of users.

Menaka: That includes scientists who are new to programming, as well as scientists who might not have powerful enough computers to run this kind of analysis,

Natalia Ivanova: Because that is an important fact. , many of these computations, they're quite heavy. So it is still kind of cutting a lot of this processing time for people, where they can submit the data and then run the analysis in the end with a few clicks of a few buttons, that’s all.

Menaka: For Elisabet, that was true.

Elisabet Perez Coronel: I was doing field work, I was doing lab work. I tried to do a little bit of bioinformatics, but I was more like a jack of all trades than like a specialist in something. So I'm grateful that those tools are available because they make the job easier for people that might be multidisciplinary and then you know, like we can all benefit. It was just fun to find out things about this.

Natalia Ivanova: It's exactly - Basically she's saying exactly what we meant, when we were developing it, right? So basically if you can do the bench science, then the processing, we can help.

Menaka: So — back to climbing the granite face of Mike and Elisabet’s data. They have lots of information from lake water where organisms are creating surprising amounts of methane, especially in treatments where they're exposed to light. They want to make comparisons across these treatments, so they upload data to the IMG website.

Mike Beman: So these were actually metatranscriptomes where we had all the genes that were expressed, and how that had changed in our different treatments compared with the control incubations.

Menaka: And to understand what's making methane, Elisabet is trying to pull out the genes that microbes express in high-methane samples. She uses one tool to see if those genes fall into specific workflows within cells — or protein pathways.

Elisabet Perez Coronel: Can I focus on photosynthesis or carbon fixation or metabolism? What is the data telling me, are these different from each other?

Menaka: What the data told her — was that photosynthesis pathways were more active in those higher methane concentrations. Whatever was making methane, was probably also doing photosynthesis, like that earlier paper had suggested.

Elisabet Perez Coronel: But it was like, so broad. It's like, what about photosynthesis is different?

Menaka: Basically, within the whole process of photosynthesis, there had to be specific functions that created methane. So she combed through this metatranscriptomic data, then lined it up with the stable isotope signature data she also had, and they arrived at their answer.

Elisabet Perez Coronel: In these lakes, methane is being produced by at least two different mechanisms, by different groups of bacteria,

Menaka: These processes create methane gas along different pathways. One process is breaking down a compound called methylphosphonate. Then, Mike and Elisabet found another function that makes methane — the pathway that metabolizes bacterial chlorophyll.

And they were also able to find specific bacteria that seemed to be contributing a lot of this methane.

Elisabet Perez Coronel: It started from kind of looking at these different phylogenetic groups that were abundant in our samples and what they might be doing. And we found that one of the ones that were most abundant is this one called Limnohabitans.

Menaka: Limnohabitans are freshwater bacteria — they were only discovered in 2010, but they live in lakes and streams around the world. And when Mike and Elisabet read up on this bacteria, they found that it could do a really weird kind of photosynthesis.

Mike Beman: So it's called aerobic anoxygenic photosynthesis, so kind of a word salad.

Menaka: So this is a special bacterial form of photosynthesis. It works differently than photosynthesis in plants, and it doesn’t run on chlorophyll. Along the way, the process seems to produce methane.

Mike Beman: So we end up with, you know, quite a few organisms that are pretty common in freshwater. They can produce methane by multiple routes. So that's, you know, again, interesting, but also really complicated.

Menaka: So that’s one little piece of the methane paradox, unparadox-ed. But the hunt for freshwater methane production routes isn’t over. This is probably a more widespread process than we’ve realized. When Mike and Elisabet compared their sequences online, they found that other researchers had sampled similar bacteria thousands of miles away.

Mike Beman: It turns out they are almost identical to some from freshwater lakes in Northeastern Canada. so these organisms are pretty common. And then if you look at the 16S sequence, they're pretty common around the world.

Menaka: And there may be other mechanisms that people haven’t even discovered yet.

Elisabet Perez Coronel: could there be something else? So maybe. Like at this point I feel like, it’s possible, so.

Menaka: And it’s good to know more – because overall, in terms of the atmosphere, unfortunately, more methane, means more problems. But Mike, and other researchers, will keep looking into this.

Mike Beman: You know, I think the next really key question is, well, how might they change in the future, right? Will they continue to produce a lot? Could go down, could go up.

Menaka: To get closer to understanding how we can limit global warming and protect these ecosystems, Mike’s group has more ideas. They might try culturing Limnohabitans and other organisms to understand them better. Or, they might focus in on genes and proteins related to making methane in freshwater.

Mike Beman: Now we've gotta look at all these different things in freshwater and figure out what's more important, where or not, and what regulates them.

Menaka: And what that really means —

Mike Beman: Always more experiments and more data, of course.

Menaka: So again, that was Mike Beman and Elisabet Perez Coronel, who published this work in Nature Communications. We'll link to their paper in our show notes. Mike is a professor at UC Merced, and Elisabet is now a technical application scientist at Thermo Fisher.

Rekha Seshadri and Natalia Ivanova shared a bit about the IMG/M database. They also hold webinars and workshops, and we'll have links in the show notes.

The JGI enabled this work via the Community Science Program. You can find out more about this work, and the Community Science Program, at the JGI website – more links in the show notes! And, the transcript will be posted online.

This episode was written, produced and hosted by me, Menaka Wilhelm. I had production help from Allison Joy, Massie Ballon, Ingrid Ockert and Graham Rutherford.

You heard music in the middle of this episode by Cliff Bueno de Mesquita, who's a multitalented postdoc at the JGI.

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Thanks for tuning in. Until next time!