Customer case study | University of São Paulo
Bruno Nozima, technical application specialist

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Services and support

An environmental scientist in South America didn’t know what to do about flawed data—until her Thermo Fisher TAS saved the day

Introduction

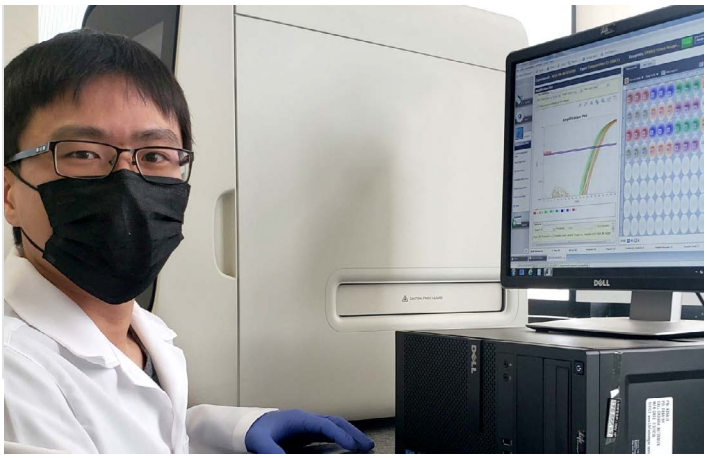
Bruno Nozima is a technical application specialist (TAS) based in São Paulo, Brazil, who started his career with Thermo Fisher Scientific in 2017. He received his PhD in Biological Sciences from the Federal University of São Paulo, where his research focused on the molecular biology of thyroid cancer.

Given the importance of his academic research, it comes as no surprise that Nozima recognizes the high stakes involved when customers reach out to the Thermo Fisher technical support team.

“They usually contact us in critical situations when the most important results of their research or procedures are at stake,” he explains. “Approaching problems with fresh eyes and backed by experience, we help our customers to find the best possible solution, aiming to deliver a great customer experience.”

This customer-centric approach extends to all of Nozima’s efforts to meet the specific needs of the researchers he supports. He points out that he makes product recommendations that not only match his customers’ workflows but also fit their budgets. And because his Latin American customers sometimes have difficulty

> "[Bruno's services and support] have saved our lab money and increased lab productivity."



Bruno Nozima, PhD, technical application specialist at Thermo Fisher

with technical content in English, Nozima is leading a project to implement a new tool that provides technical content in local languages to mitigate this challenge.

Recently, Nozima was enlisted to support Isabella Bordon, PhD, an environmental science researcher at the University of São Paulo, who had been trying to replicate an experiment. She couldn’t generate similar results and couldn’t figure out why. After extensive troubleshooting that involved analyzing publications, conducting several calls to understand the test results, and making suggestions for next steps, Nozima helped her discover flaws in the initial experiment and develop better procedures. We spoke with Bordon to find out more about how this technical support helped her overcome that challenge.

Isabella Bordon, PhD, Environmental Scientist, University of São Paulo

Can you tell us about yourself, your lab, and your research?

I'm a biologist working in environmental science at the Biomedical Institute of the University of São Paulo, Brazil. In our lab, we use multidisciplinary approaches to study freshwater and marine environments. We specialize in histological studies, inorganic chemistry, biochemical assays, and biomolecular approaches.

What challenges are you trying to address?

Transcriptional profiling of metallothionein isoforms. During my PhD research, I started studying *Callinectes danae*, a swimming crab commonly found in estuaries in Brazil. This crab is an important part of the environmental web of the estuary and for recycling of nutrients. Unfortunately, in South America, we have releases of inorganic compounds like metals into the environment. So, it is very important for ecological and environmental reasons to understand how the crab bioaccumulates these metals. There is a European species that is better understood, but for the South American crab, we do not have much information, so that's why I'm studying this species' isoforms.

We want to be able to explain how it works at the molecular level. Not just, "the crab is in the sediment, and then it bioaccumulates," without providing more information. It's an organism with its own detoxification mechanism, so we want to understand how it functions. Regarding human consumption of the crab, this has economic importance for fisheries. They transfer these organisms into clean water for consumption, so they want to know how long it takes the crab to acclimate to a clean environment. This knowledge can help fisheries estimate how many hours it takes for the crab to free itself of toxic metals.

What made you look for guidance outside of your lab?

During my first postdoc, I used spectrophotometry to measure lead bioaccumulation and metallothionein levels in the crab due to dietary and waterborne exposure. I was part of a team that published a paper on this in 2018. So that was my first step.

The second step was to understand the crab's isoforms, but I discovered through the US National Center for Biotechnology Information (NCBI) that no one had deposited the sequence of the crab's nucleotides.

That meant that in my second postdoc, I had to sequence the isoforms using Sanger sequencing, but I had no experience with PCR or interpreting the results of cDNA amplification. So, I asked some colleagues for help with this. Since I didn't know how to do PCR, they taught me how to do it, so I was following without understanding. But when a colleague of mine from another university tried to deposit the sequences they gave me, he said something was wrong; the sequences were not okay.

Can you elaborate on the problem?

There was too much variation in the results. I couldn't understand how the replicates could vary so much when they were from the same pool of samples. I couldn't understand what was causing this problem. I was completely lost, so I contacted our Thermo Fisher sales representative.

"What happened to these sequences?" I asked her. "Because the results of our research are not right." In fact, my colleagues had done the sequencing once. The Thermo Fisher rep explained that we should do more sequencing to get good results. She put me in touch with our TAS, Bruno Nozima, because he has expertise in sequencing.

What had made you choose to reach out to Thermo Fisher for help?

Our lab has an Applied Biosystems™ QuantStudio™ 3 Real-Time PCR System, and we also use Applied Biosystems™ Fast SYBR™ Green and PowerUp™ SYBR™ Master Mixes, Thermo Scientific™ plates, and Invitrogen™ reagents. So, we had a relationship already established.

How did your TAS help you?

My colleagues that had provided the sequences had already left the lab, and, as I said, I didn't understand why the cDNA amplification results were so variable. The problem was now in my hands. I was very upset. This was in 2019, right before the pandemic.

When I met Bruno, I told him how I had been taught and presented him with the results. He asked many questions about the process. He inquired about the type of crab that was subject of the study, about previous studies, as well as technical details, like plate design, extraction details, and many other questions.

I explained the problem with the replicates.

"Let me have a look," he said. "Let's see the Cts. Let's see everything."

Can you reflect on your experience of the virtual support you received from him?

Yes, he helped me use the Thermo Fisher Cloud—which is a wonderful program. In fact, congratulations to Thermo Fisher because it's an amazing tool: I was able to draw a picture to show him how the original process was designed, and then he showed me how to understand and generate better statistics. For example, he showed me the importance of replicates. When we calculate the Cts, we have to understand the variance of each of the organisms. He helped me realize that I had done something wrong in the plate design. I had been pooling the organisms to try and replicate the pool, but the right thing to do was to do replicates—separate plates—for each one of the organisms that was exposed to metals. In fact, I had made many mistakes at every step. Bruno knew that I was anxious and frustrated, but having him support and help me in troubleshooting the issue really helped me.

Can you share more about that process?

Every Friday for a month, Bruno was there to help. We did an RNA and DNA extraction. First, we did the process exactly as we'd done before—so he could show the reasons for the sequence of mistakes that had happened throughout the initial process. Then we did it the way Bruno recommended. It was much easier and a more productive use of time.

He helped me understand every step of the qPCR amplification process, from mRNA extraction to the interpretation of results, from sequencing the genome to the amplification and explanation of Cts.

One thing the researchers had told me was that the Cts have to be at "1". But these organisms that we are talking about, they were exposed to lead, cadmium, and copper, so of course we are going to have Cts higher than one. I showed Bruno that the Cts were at 22. He explained why they did not have to be at 1. All sorts of insights like that came from him.

For instance, he showed me that I could use less Invitrogen™ TRIzol™ Reagent for the RNA extraction.

In one of our weekly meetings, I said, "Okay Bruno, I'm going to extract new samples for the experiment now."

He responded, "Let's see if you really need 1 mL of the reagent because you might be using too much. Try using less as an adaptation for your sample; I think it will work."

In environmental science, we use many samples. It's not the same as researchers in molecular biology who may do two or three replicates. I have 56 animals and three types of issues. So, I adjusted the amount of TRIzol Reagent based on his recommendation and the result was amazing—the purity, the yield. Thanks to Bruno's help solving this problem, we've developed our own extraction protocol.

He also showed us how to use the geNorm test to identify and select an endogenous (housekeeping) gene from a group of candidate reference genes.

I am confident now in doing PCR, replicating my organism, and analyzing sequencing data. The reason I can talk to you regarding the whole PCR process is thanks to Bruno's help.

How were you supported beyond the weekly meetings you had that month?

I was able to chat with Bruno not just on Fridays. I would contact him during the week with questions. I would email him: "Bruno. I'm desperate. What can I do?" We also touched base every month for six months until we understood and solved each of the problems.

How would you describe his accessibility while you worked with him?

Wonderful. Sometimes I couldn't make our regular meeting on Friday because of my son's schedule, but Bruno was flexible. We'd connect through a chat app to figure out when we were both available. I remember once he finished another meeting at 6:00 p.m., and then got back to me right afterward and said, "OK, let's talk." He is this kind of guy. Very available—and it was a good thing because my research was at stake.

How would you characterize his approach to working with you?

It's a good question. When I first reached out to him, I was upset about my situation. But he was calm and supportive—and this is a difference between Bruno and other support people whom I've talked to over the years.

What plans do you have to use our solutions in the future?

We will continue to extract mRNA, produce cDNA, and do more Sanger sequencing for further research.

Another important point is: we realized that issues like these confront many other researchers in environmental science. There are not many people working on adapting these techniques for environmental science here in Brazil, so it's a great opportunity to develop a manuscript regarding quality assurance and quality control for real-time PCR specifically for environmental science. I have ideas for working with Thermo Fisher on this kind of project.

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How has this support helped increase your understanding of this organism, and how is this helping for the future?

We are closer now to achieving our main goal: to show how the peptides work for homeostasis, to know what adaptations in the organism help with the detoxification process. The isoforms do not express just because of the presence of metals; they help keep the organism functioning. What we are trying to understand is how many hours, days, or even years it takes for the crab to become clear of toxic metals. If we understand this, we can keep this organism safe for consumption, help it avoid extinction, and support ecological recycling and the web of life.

Have you introduced any of the molecular biology techniques you learned to other environmental scientists in São Paulo?

Yes, and not just for the University of São Paulo, but also for São Paulo State University. I told a supervisor I worked with there about the work I'm doing, and he said it's nice to know because we have a lot of projects that require amplification. He said we have questions about other isoforms that these techniques can help answer.

Would you recommend Thermo Fisher services and support solutions?

Yes. Bruno understood our lab's problems immediately and provided solutions as quickly as he could. We had spent a year trying to understand our mistakes, and he delivered solutions in a month. We are very thankful for his pro-activity. I'm personally grateful because many things were at play: he knows that here in Brazil, we must account for every amount of money that we spend. He knows how it works in our institution. And he knows that I'm grateful, because I've explained to representatives from the Thermo Fisher sales and technical teams how Bruno was special for us as a group and for me specifically.

I can conduct molecular biology research and talk about it with confidence now. And, since I work in an institute where nearly everybody knows about molecular biology, I feel better about my self-esteem as a scientist.

Have you stayed in contact with Bruno?

We talk sometimes regarding PCR. He said, "don't hesitate to call me," and I do call him whenever I need his advice.

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