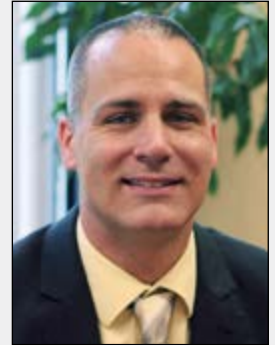


# Up Close

*with Steve Mauro*

*Microbiologist Dr. Steven Mauro, a dean at Gannon University, has dedicated more than a decade to understanding freshwater microbial communities. His work gained national attention in 2011 when he and his research team discovered that small concentrations of fluoxetine, an antidepressant, are killing *E. coli* in the nearshore waters of Lake Erie. This finding sparked a series of ongoing studies investigating how fluoxetine and other pharmaceutical chemicals, both individually and combined, are changing the bacterial communities that help keep aquatic ecosystems healthy.*

*Earlier this summer, IISG sat down with Dr. Mauro moments before he set sail on Lake Erie to discuss his research on PPCPs, what his results mean for the lake and the communities along its shores, and the importance of public outreach.*



**What sparked your interest in PPCPs?**

It was actually [Pennsylvania Sea Grant](#). They were interested in PPCPs and asked if I wanted to do some testing. It wasn't my primary area of research, but I liked the idea of trying to understand what chemicals are in the water and what they may be doing to the microbe population. It started as a side project that got really interesting.

**You started by looking at a fluoxetine, an antidepressant. Tell us about that work.**

We started by doing water sampling in areas where people swim near the shoreline at Presque Isle State Park [a peninsula near Erie, PA] and followed the standard US Environmental Protection Agency (EPA) protocol for collecting recreational water samples for microbe analysis. We were looking for *E. coli* and other microbes. At the same time, we looked at the levels of fluoxetine and other chemicals of concern using ELISA microplates.

**What are ELISA plates?**

It stands for enzyme-linked immunosorbent assay. What that means is there are molecules at the bottom of the plate that adhere to chemicals. These are very specific to the chemical you are testing, and you get a fluorescence reading based on that interaction. So, if you have a standard curve of known concentrations, you can correlate your results to that standard curve based on the fluorescence of individual samples.

**How does the process work?**

You just add a little of the water sample—100 microliters—to the plate and let it sit for a while. Then you do a few washes and put in an indicator enzyme that will allow the fluorescent reaction to occur. You do a few more washes and add the trigger, which will change the color of the solution. Then you put it in a spectrophotometer, which reads the intensity of the light wave. In the case of fluoxetine, the more yellow the solution is, the lower the concentration. The clearer it is, the higher the concentration.

As long as you have known concentrations for the chemical, you can determine the standard curve and get your results. The equipment kits actually

come with known concentrations, so you can create your curve and make sure that all of your samples fall within the range of that curve. This is an interesting and important area of research, and the biotech companies have picked up on it.

**What concentrations of fluoxetine did you find?**

We found it at really low levels, below what's been shown to cause to harm to aquatic species in studies done so far. But the interesting thing is that, while those levels were low, they were consistent. Fluoxetine was frequently present in low concentrations.

We did discover in some follow-up studies that other chemicals of concern can act synergistically with fluoxetine. They can actually exacerbate or increase the effect it might have on *E. coli* and other microbes.

**Which chemicals?**

We have looked at triclosan, an antibacterial commonly found in household products, diuron, an herbicide, and estradiol, which is a synthetic hormone. We picked chemicals that would allow us to gauge household vs. agricultural sources of water contamination. There is also a lot of research on triclosan. It is an established chemical that has been analyzed a lot, so we thought we would be able to more readily understand the effects of mixing it with fluoxetine.

In one of these studies, we exposed *E. coli* to a mixture of very low concentrations of fluoxetine and triclosan. When those chemicals were combined in low concentrations, they actually had an effect on the microbe population. Together they appear to damage and kill *E. coli*, whereas alone they have no effect.

**Do you know why that is?**

We don't yet. We have some pretty strong evidence that fluoxetine can act as a DNA-damaging agent. For example, we found that fluoxetine can induce what is called the SOS DNA damage response in *E. coli*. This can actually trigger cell death by reactivating latent viruses. Our lab studies of *E. coli* have shown that fluoxetine reactivates latent viruses that are pathogenic to humans. The thought is that it may just be generally damaging the bacteria's DNA. Triclosan might be just increasing that overall damage to the cell.

**Why test the impacts on *E. coli* specifically?**

*E. coli* is the main indicator used to gauge water quality in freshwater. We have thresholds in place that are good predictors of gastrointestinal illness. If the chemicals are killing *E. coli*, we might think the water is safe for swimming when in fact it is laden with other chemicals and bacteria that might be dangerous.

Also, there are actually many other good bacteria in the water that are supposed to be there. These are often nutrient-recycling microbes. If fluoxetine and triclosan have the same effect on these bacteria—that could be very bad. And given the mechanism of DNA damage, it is very likely that other bacteria are impacted as well.

**How did you measure the impact?**

The field actually takes a very comprehensive approach to this now. We grow it on plates, just like you would in a high school or college microbiology lab. The plates are called modified m-TEC plates, which have nutrients in them that help *E. coli* grow at a specific temperature. All the other bacteria die, and you are left with purple colored *E. coli* colonies on the plate.

Then we use an EPA protocol to count the number of *E. coli* and relate it back to a threshold correlated with gastrointestinal illness.

But the really neat thing we did was isolate bacterial DNA from the water samples. We took bulk water samples, isolated the DNA, and then sequenced part of the DNA of all the bacteria present. So, you can identify every bacteria in the water from one sample. This allows you to study the population genetics of the bacteria in a way that we were never able to do before.

**What does this tell you that previous methods didn't?**

It not only tells you how much *E. coli* you have in the water, it also tells you what other bacteria are there and in what quantities. We know there are some bacteria that should be in the water, like cyanobacteria. If you don't see that bacteria, you know something is wrong with your water sample. If you see all the other fecal bacteria, that is an indicator that something is not quite right in the water.

It also gives us an idea of the overall impact the chemicals have. Imagine you had a water sample. You can divide that in half and treat one half with fluoxetine, triclosan, and other chemicals of concern. Then you can compare it to the half you didn't treat to see how these chemicals actually changed the entire bacterial composition of the water sample.

**Is the potency of fluoxetine and triclosan together enough to harm larger aquatic species?**

We haven't looked at other organisms yet. But changing something at the primary level—microbes—changes things all the way up the trophic levels. Small changes in microbe populations could lead to changes in protist populations [the hunters and grazers of the microbe world], for example, which could in turn change fish populations. We don't know yet what direct impact these chemicals have on other organisms at the levels we are seeing. But they could have an effect on the entire food chain indirectly if they impact the primary microbes.

**Going back to your original study, are there any direct sewer or wastewater treatment plant outputs near Presque Isle State Park?**

No, there aren't. Our wastewater treatment plant is to the east of the park, and the water generally flows from west to east. We don't have any direct wastewater treatment plants in the area that would be impacting the beachfront where we were sampling.

**Where did the fluoxetine come from, then?**

Those might just be the concentrations that are generally in the lake. We will actually know more about this in the near future. We are going out a little farther into the lake soon to test the water. I am taking a few students on the *US Brig Niagara* for a few days, and we are going to be sampling deeper out in Lake Erie. Then we are doing an *R/V Lake Guardian* cruise through the EPA with high school teachers, and we will be testing a bunch of different areas across the lake.

**Have you looked at fluoxetine concentrations in other lakes?**

I did a little work in Edinboro Lake, which is a little bit south of Erie. It is a smaller, contained lake. We are also partnering with [Dr. Gerald Koudelka](#) at the University of Buffalo, and he has been doing some work on Lake Ontario.

**Does Lake Ontario have the same ambient concentrations? Would you expect it to?**

It is too early to tell. We are still in the sample-processing stage. I would anticipate that this is a general effect across all the lakes, though. The streams and creeks could have runoff that is not treated. Wastewater

treatment plants could not be fully removing the chemicals. Everything indicates that this is going to be a general phenomenon across all the Great Lakes.

**How about in Edinboro Lake?**

We haven't really looked at the chemicals of concern there, but we have a good understanding of the bacterial pollution.

**What is the difference in bacterial populations in smaller lakes vs. the Great Lakes?**

It depends on the lake. It depends on the day, really—on what is flowing in on any given day. Generally, though, small bodies of water, especially streams, creeks, and smaller lakes, have much higher concentrations of *E. coli* and other fecal bacteria.

**You mentioned you will be testing farther into Lake Erie soon. You are actually starting that work today, correct?**

Yes. I am about to take off on the *US Brig Niagara*, and we are going to be doing chemical testing at various locations. I am just going from Erie to Cleveland, so I will get a broader spectrum of samples for at least that small range. Later in July, when I am on the *Lake Guardian*, we will be sampling all across Lake Erie at about 15-20 different locations.

**How far offshore will you be testing?**

We will be quite a distance offshore, in the middle of the lake in some instances.

**Is it your class you are going with on the *Niagara*?**

It is not my class, no. It is actually part of a consortium being led by Dr. William Edwards this year. He is a faculty member at Niagara University. There are different professors from across the region who take part in this course each year, and they recruit some of their students as well. Overall, it is a three-week journey around a couple of the Great Lakes. I am just doing one leg of it.

It's worth mentioning that Gannon University has established a new freshwater and marine biology major, so I anticipate doing more sampling trips with more students in the near future.

**What will you be testing?**

I will be looking for chemicals of concern like fluoxetine using those ELISA plates again.

I will also be trying to filter microbes from the water samples to isolate their DNA and determine which bacteria and virus are present at the different locations. This is a follow-up to other work I have been doing. We will be able to filter and process the samples on the boat. The overall analysis will occur back in the lab, but we will at least be able to get to the point where the samples are ready for analysis.

**Are you just taking water samples, or will you be doing sediment sampling as well?**

We will take a few sediment samples, but the specific microbes we are looking for will be on the surface of the water.

**Do you expect the samples from farther out in the lake to look different than your nearshore ones?**

It is going to be really interesting. I am trying to keep an open mind. I would expect to see lower concentrations. You would expect there to be higher concentrations along the shoreline, where things get deposited into the lake. But as you move out to the middle of the lake, for example, and have a more diluted sample, you would expect to see a lot lower concentrations. That's what I would anticipate. That is what I am hoping for, actually. We really don't want to see these things out there. But that is why we do the science—to see what is actually there.

**Will the work on the *Lake Guardian* be similar?**

It will be similar, but with more samples because there will be more sample sites. And we will be involving high school teachers. The idea is to show them what we do so that they can go back to the classroom and either do it with their students or at least teach them the basic principles for things they will hopefully learn in college.

**Have you done a research cruise like this before?**

No. My work so far has been in nearshore waters. Gannon University has the Environaut, which is a 53-foot research vessel. That is small, and we don't go out that far. The most I have done is day cruises. To be able to spend several nights or up to a week on a research cruise is a new experience for me.

**Have you worked with teachers in the past?**

I have. Pennsylvania Sea Grant has been great for that. We also have the Regional Science Consortium here, which brings in busloads of students all throughout the summer. I usually plug into ongoing events they have and do different types of lectures or demos.

**Do you enjoy working with teachers?**

I do. It's fun. And it's important. It is really important. College professors should be talking with high school teachers so they know what it is we want students to be prepared for. And we also need to know what they are doing at the high school level so we can adjust accordingly.

Erie is a great place for this sort of work with Pennsylvania Sea Grant and the science consortium here. We also have a really good community with the Department of Environmental Protection, the Department of Conservation and Natural Resources, and other local organizations that really work together. They integrate the universities into the work they do well.

**What advice do you have for other researchers who are interested in doing outreach work like this?**

My advice is to have patience. It is not going to be everyone's main interest. When you have groups and groups of high school students, you have to realize that not everyone is going to love this stuff as much as you do. Also, teach it in a way that is understandable to someone who knows nothing about the subject. Make it fun and interesting. I really think it is. I think the students can get a lot out of it and gain an interest where there wasn't one before if the outreach is done right.

I mostly look at bacteria and viruses in the environment to try to understand what they are, what they do there, and whether they are supposed to be there. Our first PPCP study was a side project that became more because we were really interested to see how these chemicals influenced bacteria and viruses.

**What else does your lab work on?**

My lab is also trying to reduce the turnover time between collecting *E. coli* samples and determining whether the water is safe to swim in. You have to plate *E. coli*, and that can take 24 hours, which means you can only tell the public that the water was safe to swim in 24 hours ago. We have been working with a statistician at Penn State Erie, the US Geological Survey, and the Regional Science Consortium. We have been working with predictive models that use readily and immediately available weather conditions and other factors to anticipate what *E. coli* levels will be the following day. These models can be run in real time, so you get results right away. For my part, I am using qPCR [a biochemical technology used to replicate DNA], which you can look at in 2 hours instead of 24.

We are actually employing this at Presque Isle State Park now and are able to predict water quality there in near-real time. We can send out warnings—and the beach manager does—if the models and qPCR predict high *E. coli* levels. This project has been fantastic and has been moving along great.

The other thing I do is look at pathogenic microbes in the environment. The one I focus more on is Shiga toxin-producing *E. coli*, which is a particularly pathogenic strain. We were trying to see if it is present in recreational waters. Long story short—it is. It is very sporadic in its distribution, but it is there. Now we are trying to understand the factors that might cause it to be somewhere and at what levels.

**What is Shiga toxin?**

It's a toxin that basically causes cell death when it is released into your bloodstream. It is very bad. As far as bacterial infections go, it is the number one cause of acute renal failure in children under the age of five. There are 10,000 or so infections a year in the US, but it's really a worldwide problem. You can think of it as akin to traveler's diarrhea. Those are essentially the symptoms it causes.

We were looking to see if that strain of bacteria is present at Presque Beach to get a better handle on whether we actually have things in the water that can cause gastrointestinal illness. And we do, but we see it only sporadically, and it is very hard to predict whether it is present or not. Our main contribution to this work—again done in collaboration with Dr. Koudelka—was showing that this strain of bacteria actually kills grazing protists, so it is very hard for the natural predators that would tend to eat and destroy this bacterium to get rid of it.

**Is this strain present with other *E. coli* strains?**

No, and that is why we think that the work showing that it kills protists is an important finding. General levels of *E. coli* do not predict whether the Shiga toxin-producing *E. coli* will be there or not. The reason, we think, is that general *E. coli* is killed by protists, but Shiga toxin-producing *E. coli* is not.

This demonstrates the need for taking a look at pathogenic microbes in addition to the overall indicator species. Sometimes the indicator is predictive of what is in the water that might lead to illness, but sometimes it's not. As we develop new technologies and get more information, we should always consider how we are going to use that information to gauge water quality and safety.

**What else do you want people to know about PPCPs and their impacts on microbes?**

One thing I would want people to know is that there is a way to get rid of prescriptions and personal care products, and it's not flushing them down your toilet. That is how they get out in the environment in the first place. A lot of communities and pharmacies have collection programs where you can drop off your pharmaceuticals. The other thing is, for personal care products at least, minimize your use of these products wherever you can. It really does help the environment.

I also think the general public is our greatest source for proactively researching these chemicals. If you live near a body of water and are interested in this kind of stuff, it is actually easy to collect your own water

samples. It is pretty affordable, too. We actually established an institute at Gannon University this year called Water Education Testing and Training (WETT) that does this kind of testing routinely. You can collect a water sample as an environmental citizen, send it somewhere for processing, and then learn what is in your backyard. This is always something worth thinking about. And if you have any water quality meetings in your area, or if there are groups that are cleaning up a local creek or something like that, it is always worth being a part of those.

**Where can people get the equipment needed to collect a sample and send it in for analysis?**

Really all you need is a glass vial. Just put it through a dishwasher and then boil it in water to remove any detergent and sterilize it. That will be just fine. And, like I said, Gannon University does this kind of testing routinely, so they could send it here for analysis.



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