Dr. John Kelly, a microbiologist at Loyola University Chicago, is working to shine a light on the impacts that human activities have on aquatic microbial communities, especially those that make their homes in rivers or lakes. His research over the last decade has looked at everything from how bacterial communities are affected by atmospheric carbon dioxide to the role biofilms may play in helping dangerous pathogens survive in the pipes that deliver drinking water. In more recent years, Kelly has teamed up with researchers at Northwestern University and the Cary Institute of Ecosystem Studies to examine how freshwater bacteria are impacted by pharmaceuticals and other consumer products. One of these projects, funded by Illinois-Indiana Sea Grant, is among the first to study how bacterial communities respond to a nanomaterial commonly used in cosmetics and sunscreen. The results of this study and ongoing nanomaterials research could help guide future development of these chemicals while their levels in the environment are still low.

IISG sat down with Dr. Kelly to get a closer look at the complex world of bacteria and learn more about how materials we use everyday are affecting the microbes that keep things running smoothly in aquatic ecosystems.

**How did you become interested in pharmaceutical and nanomaterial contamination?**

I am a microbial ecologist, so my interest is in exploring the composition and function of bacterial communities in the environment—what kinds of species are present in a community, and how does that community function? I came to Loyola in 2001 and have since become more interested in freshwater ecosystems like rivers and streams. Living in a really urban area like Chicago, there are a lot of issues related to urbanization and how it affects rivers and stream ecosystems. When you start looking at urbanization, it brings to mind a lot of pollutants that are present.

I had a couple of colleagues who kind of sparked my interest in both of those topics. I work with Kimberly Gray at Northwestern, who is an expert on nanomaterials. She is an environmental engineer, and she has been working for about a decade or more on nanomaterials—she specifically works with nanotitanium dioxide, which is one of the most widely commercialized of these engineered nanomaterials. She has been working for a long time on the development side, so she knows how to manufacture the materials. She knows all the properties. But very few people have investigated their potential environmental effects. So, because of my interest in the way that bacteria in streams and rivers function, it seemed like a natural pairing.
As for the pharmaceuticals, I got interested in that from the literature, from reading things both in the popular press and scientific literature about how they are finding all these pharmaceuticals in water all over the world. I have another collaborator, Emma Rosi-Marshall, a faculty member at the Cary Institute of Ecosystem Studies in New York. She is very passionate about pharmaceuticals and emerging pollutants. She is a stream ecologist, and I am a microbiologist, so it was another nice pairing. She suggested some projects, and it just sort of came together.

In terms of the nanomaterials, we have only focused on one, nanotitanium dioxide. There are two reasons for that. One is that it is one of the most widely commercialized. This material hasn’t reached high concentrations in the environment yet, but it is used in a wide range of industrial and commercial products. If your question is whether there is going to be an environmental impact of nanomaterials, you might as well start looking at one that is widely used. Two, like I mentioned, my collaborator is an expert on these materials. Nanotitanium dioxide is a very interesting material. It comes in different mineral phases that have different properties, and it can be made into different kinds of particles, both different size particles and different shapes. My collaborator has one of her students working on building different shaped particles—flat sheets or hollow cylinders or very, very thin wires. And they seem to have different toxicities. We have been testing their toxicities to bacteria, and they seem to be behaving differently.

Yes. This is very interesting. Because you can do so many different things with these materials, it makes them very interesting. That is why we chose the nanotitanium.

For the pharmaceuticals—there is such a huge range of those things. Some of what we chose came from the literature. The U.S. Geological Survey did a big study a few years ago where they surveyed 139 streams across the U.S. and looked at about 95 different contaminants. We went to that list and said “What are the most commonly occurring ones? What are in the highest concentrations?” We also like to try to pick pharmaceuticals that are really different from each other in terms of their structure or how they interact with biological systems. It would be less interesting to me if we just picked a whole set of antibiotics that all have the same target in bacteria. We are picking different kinds of drugs that have really different modes of action and have different structures to get a sense of what the potential impacts might be.

We are interested in trying to see what kind of effects different structures might have on the bacteria. We are mainly picking compounds that aren’t designed to interact with bacteria at all. We use antibiotics to kill bacteria. Most of those are naturally produced—some are chemically synthesized—but the goal is to kill bacteria. Many of the drugs that we take, though, are not designed to target bacteria, but they interact with biological systems in some way. That is why we use them. They are not designed to target bacteria, but if you expose bacteria to these compounds, will anything happen? So, we have been doing studies with things like caffeine, antihistamines, anti-convulsive drugs, and anti-diabetic drugs. These are things that people take a lot of, but we don’t know if they are going to have any effect on bacteria. That is why we picked those.
Yes, for some we have. We had a paper that came out earlier this year where we tested a bunch of different pharmaceutical compounds. We saw some interesting effects with some of the antihistamines, both antihistamines taken for allergies and those taken for indigestion. Some of those had really significant effects, especially on algal primary production. They also reduced bacterial respiration and shifted the composition of bacterial communities. That is an example of a drug where we don’t know why it has this effect. Now we can investigate it further and look at its biochemistry, but in the beginning we were just screening to see if some of these drugs have effects.

Essentially it means that they are just less active. They are not as healthy. Respiration is one of the main modes of metabolism that you see in bacteria. If you see less of that, that is showing a negative effect on the community. Often times it is followed up with fewer bacteria, so you are killing a lot of them by having some kind of negative effect on their metabolism.

We also did a study where we focused on an antibacterial agent called triclosan. We don’t ingest triclosan to treat infection, but it is used in a lot of soaps, toothpastes, laundry detergents, and cleaning agents—it is a really widely commercialized compound. It is very frequently found in rivers and streams in urban areas. We are very interested in bacteria that live in sediment, so we focused on triclosan for that study because we hypothesized that it would end up there. It is not very soluble, so whatever goes into the stream is going to end up in the sediment. And other studies have shown that once it is in sediment it doesn’t degrade very quickly. It hangs around for a long time. Our thinking was, even if the input of triclosan is low, if it gets into the sediment it could build up over time and reach pretty high concentrations.

We did two different studies. For one of them, which was recently published, we chose two rivers in the Chicago metropolitan region. One was the Chicago River, which runs right through the city of Chicago, and the other one was the Des Plaines River, which is out in DuPage County in the suburbs. Two rivers in very different habitats—highly urbanized vs. suburban. They both receive direct input from wastewater treatment plants. For that study, we did sampling upstream and downstream of those two treatment plants to look at how treatment plant effluent might affect bacterial communities. What we saw, which was really interesting, is that the sites that were downstream had higher levels of inorganic nutrients, more nitrogen and more phosphorus. In all the other studies I have seen, increasing nitrogen and phosphorus in a stream—whether through effluent, fertilizer application and runoff, or anything else that humans do to add nitrogen and phosphorus—tends to stimulate microbial development. So you would think that there would be more bacteria. But in our study, when you went downstream where there were more nutrients, there were significantly fewer bacteria. We also looked at the species composition and saw a significant decrease in diversity. As you go downstream of the effluent, there are fewer bacteria, and the communities are less diverse in terms of the number and types of species there. If the nutrients are going in, that should help the bacteria. There must be something else in there that we didn’t measure, like an emerging pollutant that is having a negative effect on the bacteria. And then we wanted to investigate if maybe that was triclosan.
So, we had those two sites, and then we had a site way out in McHenry County, which is a much less urban area. It is near a state park and there are no wastewater effluent inputs into that stream at all. When we measured the triclosan concentrations, they were much, much higher at the urban site, still measurable at the suburban site, and then below detection at the McHenry County site. So there was a strong gradient of urbanization effect on triclosan concentrations. But when we looked above and below the treatment plants, the triclosan was actually higher upstream than downstream for the urban site, the north part of the Chicago River. That is where we saw our highest concentration. What we realized later was that there is a lot of combined sewer overflow inputs into that river above the treatment plant. It seems, for triclosan at least, the main culprit in Chicago is the combined sewer overflow, not the wastewater inputs. Treatment plants remove triclosan pretty well, but when there is high rainfall, sewers release water directly into the river without treatment. The triclosan just goes into the water and can get into the sediment and accumulate there.

**What impact did you see to bacteria where triclosan was at its highest?**

We saw that there was a significant correlation between the triclosan concentration in the sediments and the bacterial community’s resistance to triclosan. Where you had communities that were exposed to higher levels of triclosan, a higher percentage of those bacteria were triclosan resistant, which makes perfect sense. But, prior to this, nobody had really documented an effect of triclosan in the environment. We showed that it does seem like it is having an actual effect. It is enriching for bacteria that are more resistant to triclosan.

**What does that resistance mean for the ecosystem?**

One reason that is a concern is that there have been a number of studies linking triclosan resistance to resistance to other chemotherapeutically useful antibiotics. In other words, we don’t use triclosan to treat disease if we get sick. We use it to clean things, but we don’t use it to treat disease. But there are a lot of other antibiotics that we do use to treat diseases. What the literature suggests is that if bacteria develop resistance to triclosan, they can be resistant to other antibiotics that we use to treat diseases. The concern is that we are in some ways enriching for antibiotic resistant bacteria by using so much triclosan and putting it out in the environment.

**When we talk about developing resistance, are we talking about bacteria that were already less sensitive to these chemicals or ones that became less sensitive overtime?**

That is a really good question. We don’t have definitive evidence about that yet. We are talking about community resistance, so how many bacteria in the population are resistant. If you have a community that is exposed to triclosan and its level of resistance goes up, a couple of things could be happening. One is that the bacteria that were already more resistant could be proliferating and sensitive ones could be dying. The other possibility is that the bacteria that are there could be developing resistance via mutation or via horizontal gene transfer. If what we saw was a death of sensitive ones and an increase in resistant ones, then that should be reflected in some kind of change in species composition because that would mean that the species mix is changing.

We couldn’t really tease that out in the field study because when you are talking about the Chicago River, the Des Plains River, and McHenry County, there are so many other variables, so many differences, that it would be impossible to say that this changes because of triclosan.
But we also did an artificial stream study in the lab where we set up model streams with sediment and flowing water, and we exposed those to triclosan. When we did that in the lab, we saw a huge increase in community resistance and a shift in species composition. The species changed between the triclosan-exposed streams and the clean streams. That doesn’t definitively prove that is why they are becoming more resistant. They could still be developing mutations or transferring resistance genes, so there are still a lot of questions to answer about that.

I would think that if the resistance is coming about through mutations or gene transfer, that would have less of an effect on the function of the community. That would mean the species composition doesn’t really have to change. But, if the shift is happening because the ones that are sensitive are all dying and the ones that are resistant are all growing more, I think that could be more problematic because then you are changing the composition of the community, and that could potentially change the function. But we don’t have data to nail that down.

You would have to design an experiment around that particular issue. So, triclosan targets a particular enzyme in bacteria, an enzyme that is involved in the synthesis of lipids. People have identified the gene for that enzyme and its sequence. One way to develop resistance is for bacteria to develop mutations in that gene. They have shown this in the lab, for example, in *E. coli*. They can raise *E. coli*, expose it to triclosan, and it will develop mutations in that gene. What happens then is that the enzyme structure changes a little and the triclosan won’t bind anymore. We actually could do an experiment where we look at that gene in particular with the data we have right now. We have the DNA. We could sequence that gene before and after exposure or for the control vs. treatment bacteria and see if there are differences in the sequences.

Another method of resistance is what is called efflux pumps. These are just what they sound like: pumps that bacteria use to pump out triclosan and antibiotics. Some of those have been identified, so you could also look for the genes that encode those pumps. We could also specifically look for those genes within our community and see if the frequency of those genes is changing with triclosan exposure. Is that what’s driving the resistance?

Yes. One of the things that is interesting about nanomaterials is that we think they could have some significant effects on the ecosystem, but they are not present at very high concentrations yet. We haven’t polluted the environment with them too much so far. For example, there are no field sites that you can go to where there is a large nanomaterial spill, like you could if you were interested in something like PCBs. We are hoping to head off some kind of future problem by figuring out what the potential impacts could be.

You can’t really do field projects for this. You have to use model systems, so we set up artificial streams with sediment that we inoculated with bacteria from the environment and left the bacteria to grow. Then half the streams got hit with nanotitanium and half didn’t. What we thought
would happen is that the titanium would kill some of the bacteria, so the numbers would go down. And we thought that bacterial activity—things like respiration and denitrification, two of the most important bacterial metabolic processes in streams—would also go down and that the species composition might change. What we saw in the artificial stream study was that it did indeed lower the bacterial abundance when we added the nanomaterial, although only for a short time. But it actually stimulated more metabolic activities. There was more respiration and more denitrification happening when we added the nanotitanium. It killed a lot of the bacteria, but the ones that were still there became more active. That was a surprise to us, but we have some hypothesis about why that might be the case.

For this study, we just did a one-time application to nanotitanium because we wanted to do what was the simplest for our first study. What we saw was that the bacterial numbers went down for about three weeks, and then by the fourth week they had rebounded back to where they were at the beginning. And when we had that decrease in bacteria, the species composition definitely changed. But then when the community rebounded by around week four, the species composition went right back to where it was before. We think that that is because the nanotitanium itself is not as toxic over time. Nanotitanium is toxic when it is illuminated, when it is hit with light, because it produces these reactive oxygen species. If the titanium gets buried in the sediment over time, or if it gets coated with organic material, that is going to limit its exposure to light. I think that is probably what happened in our model streams. Over those few weeks, it gradually got buried or covered with organic material and wasn’t as photoactive anymore. In this case, it doesn’t seem like the bacteria were developing any kind of resistance. I think it was just that the nanotitanium was not as effective over time.

There are two basic methods that we use to count bacteria. One is a direct microscopic count, where you look at them under the microscope and just count the number of cells that you see. The other one is what is called a plate count, where you actually grow the bacteria on petri dishes and count the number of bacteria that grow. Both of those methods have pluses and minus. I think that the direct count data is a little bit more reliable because you are not relying on growth. You are just counting ones that you see. When you are doing the plate count assay, you are relying on the bacteria to grow in the petri dishes. Lots of scientists have shown that only about 1 percent of the bacteria in the environment will actually grow on a petri plate anyway, so you are a getting a very small sample size. I don’t feel like the plate count data [what the report shows] is as reliable as the direct count data that we have and are putting into the paper that we are trying to write.

And one challenge with these artificial streams—they are essentially 4-meter-long recirculating streams. They are made out of fiber glass and there is water that is moved with a paddle wheel. And they are in a greenhouse. Our hope is that if we let these streams go for a few months at the beginning, the bacterial community will stabilize. Then we could do our treatment and see what kind of effect it has. The problem that we run into is that they don’t really stabilize after two or three months. All the streams fluctuate a little bit in terms of the amount of bacteria, even the
controls. Because it is in a greenhouse, if they get a lot of sunlight over a couple of days there could be more algal activity. If it was cloudy for several days, there could be less. I don’t know if it is something about those environmental conditions or just the inherent variability in a biological system, but they don’t really get that stable. It makes it challenging to try to do these kinds of experiments.

That is a good big picture question. I would argue that there isn’t really a climax community [steady state] for bacteria because their generation time is so short. They can reproduce and double their population size in the order of half an hour to an hour, so their populations can be changing so quickly. And they respond so much to environmental stimuli. Bacterial communities are really diverse. One gram of soil, which is about 1 teaspoon, has about 10 billion bacterial cells and somewhere in the neighborhood of 4,000 species. And they are all competing with each other all the time, for everything. So bacterial communities are known to sort of go through these boom-and-bust cycles. If a little piece of organic material falls in the soil, the bacteria that can eat that will grow rapidly in cell number while the other ones go down. And then they eat up all that food and die and get eaten by some other organism. There is a lot of shifting going on all the time in bacterial communities.

I don’t think it is the best model for the real world. It is a model for what might happen, for example, if there was a spill of this material. Nanotitanium is used a lot in consumer products, things like makeup and sunscreen and even some foods. So it will be released into the wastewater stream and will end up at a treatment plant before going out into the stream. But, like I mentioned before, if you have a big event and there is a combined sewer overflow release and nanotitanium goes out untreated, then you potentially could have nanotitanium coming in at a higher concentration. We picked 1 milligram per liter as our dose in this study because that is a level that has been measured in wastewater. They haven’t measured 1 milligram per liter in stream or river water anywhere, but they have found it in wastewater. If there was an event where the wastewater got released untreated, or if there was a failure in the treatment plant or something like that—if it got released untreated, that is the kind of concentration we would see. What was interesting about what we saw is that the effects were temporary. They lasted only a couple of weeks. I think that is an interesting model for what might happen in the environment if there was an untreated release of wastewater containing this nanomaterial. You would see an effect, but it would be short lived.

Nobody had done this kind of work in a complex system like a stream before, or even a model stream. Most of the work on titanium has been done in the lab, either in a flask, test tube, or petri dish. We didn’t really know what was going to happen when we put it into a stream. These issues about it getting buried or coated with organics or how much it is going to clump was really a big question for us. We just weren’t sure what kind of dose would give us any effect. This experiment has been very helpful for us because now we know that 1 milligram per liter will give us an effect. So, maybe if we start with a lower, more drawn out dose—maybe we have 1 milligram as our eventual target, and we start out with something that is a tenth or a hundredth of that that we apply every day until we get to 1 milligram per liter and then see what happens when we
get there. So, I think it is a model for some scenarios, but not the typical scenarios. But it will be informative for future experiments.

There are still a lot of questions about nanotitanium getting buried, coated, or clumping. Nanomaterials are interesting because they are so small. These things have to have one dimension less than 100 nanometers. The reason that being small is important is that they have more surface area, and more surface area makes them more chemically reactive. They collect more light, they get excited, and release these reactive oxygen species.

When nanotitanium particles are manufactured they can be in the range of 20-50 nanometers in diameter. But they sometimes stick together. And if they clump until the clump is now more than 100 nanometers across, then how does that behave? If you have one big particle, and a nanoparticle, and nanoparticles that have clumped together, how do those different things behave? We still have a lot of questions about that. The assumption in the literature has always been that if you take the small nanomaterials and they clump to the point where the clump is no longer nanoscale, then it won’t behave nano anymore. It will behave like the bulk material. It will lose its nano properties. But we have done some experiments—not in the artificial stream, but in the lab using natural stream water in smaller micro-well experiments—that have shown that even when they clump they can still be fairly active and toxic, which contradicts the central theory about this stuff. When we take the nanomaterials and put them into stream water in a micro-well plate, they will clump and make particles about 300 nanometers across, but those things are still photoactive and toxic. This data was recently published.

Probably not. The modes of action for a lot of these things are really different. For example, carbon nanotubes and carbon nanomaterials mostly have physical effects on cells. They will puncture a cell, or they can be small enough to get inside a cell and disrupt things. For nanotitanium, its real toxicity comes from photo activity. When it is hit with light it produces these very short-lived reactive oxygen species, which are highly reactive and will oxidize basically anything they come in contact with. But they last a very, very short time. That feature of the nanotitanium is sort of—I don’t want to say it is unique, but most of the other engineered nanomaterials don’t behave that way. That isn’t their mode of action, so I think the results are mostly only relevant to nanotitanium.

But, in my lab and in Dr. Gray’s lab at Northwestern, we are using another platform to study the effects of nanotitanium using high throughput screening. In these experiments, we are using micro-well plates, where you can run replicated experiments. We use robotics to basically run the experiment. You set up your plate, you set up your solutions, you tell the robot “I want this much of each of these things in these wells,” you expose it to light, and then look at it in a plate reader. That format can be used to do really well replicated experiments on lots of different things. We could pick different forms of nanotitanium at different concentrations and test all of them against representative bacteria to see which ones are the most toxic. Our hope is that by looking at different mineral phases, shapes, and sizes of nanotitanium—can we make any rules? Can we say that nanotitanium with this shape or this size or this mineral concentration is more or less toxic? The dream scenario is that we then could inform the development side. There are scientists working all over the world

Given the importance of shape and size in determining toxicity, is it possible to predict the toxicity of nanomaterials other than nanotitanium with this data?
developing all kinds of new ways to use these nanomaterials. If we could
tell them in advance “If you make them look like this, it will be super
toxic, but if you make them look like this it won’t be as toxic,” we could
potentially avoid some future environmental effects. I think that the high
throughput approach will be really useful for trying to develop some rules
about shapes, sizes, mineral phases, and things like that.

Where are you in that process?

We have one paper that we published this year in Water Research where
we show the effect of different types of nanotitanium—several commercial
ones and several that we made in the lab—on E. coli.

They have known for a long, long time that nanotitanium kills bacteria. That
is part of why it is used. It kills any kind of cell. If you illuminate it, these
reactive oxygen species will kill any cell that they are anywhere near. But all
the testing was always done in the lab. They would test bacteria in distilled
water or in the media you would use to grow organisms in the lab. But that
has nothing to do with what would happen in a stream. Stream water is
not at all like distilled water. And it is not at all like growth media. Growth
media usually has really high organic carbon content, much higher than a
stream, and distilled water has none. And a stream has a lot of other stuff
going on. What is the ionic strength? What is the pH? What is the organic
content? So, in the first paper we published, we basically took E. coli,
three or four nanomaterials, and used real water—we went out and got
Lake Michigan water and stream water—and tested the bacteria against
these different nanomaterials in natural water and saw what the effect
was. We also examined what happened if we changed the organic carbon
content. We used fulvic acids that you can buy and applied those to the
water and saw how they affect the toxicity.

We also did another study, which we have in review right now, where we
examined a set of different commercially available nanomaterials and their
effect on four different bacteria. Because, again, the thing about testing
nanomaterials is that everyone has done it mostly in the lab, either in
distilled water or media, and they have done it almost always on E. coli.
That is fine. E. coli is a good model bacteria, but it doesn’t behave like all
the bacteria. So we tested four different bacteria that weren’t E. coli to test
their responses. The interesting thing was that they responded to the same
dose of nanotitanium very differently.

What does it mean for aquatic environments when bacteria
composition and function shift
in response to contaminants?

I think it means a number of things. Bacteria catalyze a number of really
important bio-geochemical cycles in the environment. They are the
primary drivers of the nitrogen cycle. And, in aquatic systems, they are
very important drivers of the carbon cycle. If we do things to knock out
particular bacterial groups or damage them or depress all their activity, it
is going to affect nutrient cycling. So if you have a situation where you kill
large numbers of the bacteria, it is going to mean less nutrient cycling,
which is going to be bad for organisms all through the ecosystem.

A question that comes up, which I think is really interesting, and there isn’t
a lot of data on it so far, is—let’s say you have two bacterial communities.
One is in a stream that is pretty clean, and one that is in an urban stream.
So, the bacteria that are in the urban stream, let’s say they are exposed to
a constant dosing of some toxic pollutant—antibiotics or heavy metals, or
all of those things, probably. But the urban stream has been exposed to
them for a long, long time. It is functioning and the bacteria are there and doing their thing. Then you have the bacteria in the clean habitat. What happens if some new pollutant comes in? Something that neither of the communities has seen before. Is the community in the urban system going to be more resistant to a new stressor because they are tougher, they are urban, they are badass already? Or, are they so on the brink, are they so stressed out already, that anything new that comes is going to be hard to deal with? That is a question that we are interested in trying to explore. No one has really answered that question so far.

There is literature on this sort of topic in macroorganisms, but not really in microbial ecology. In macroecology, what they see is that if you have biological communities that are under stress and are less diverse, that community will be less able to withstand a new stressor. The diversity of a biological community gives it some resilience to environmental stressors. If something gets hit harder, other things can rebound, and it can reach a steady state. But if it is already less diverse, it can often not handle a new stressor as well. There is data for that in the macroecology literature, but not much in the microbial ecology literature.

I think that the nanomaterials are an interesting way to get at that question, because nanomaterials aren’t in the environment in very high concentrations yet. There are some, and there are going to be more and more, but there hasn’t been a huge spill of nanomaterials so far. It is sort of a novel stressor in a way. It is not antibiotics, it is not heavy metals, it is different. And since they are going to be coming into the environment because of human use, it is much more likely that they are going to end up in an urban environment than out in the middle of Iowa because there isn’t enough human population density there. So the communities that are already stressed are going to have to deal with another stressor. How are they going to respond to that? That is a question that we are interested in trying to explore.

I am also interested in wastewater treatment plant effluent, just as a broad anthropogenic input. We use so much water in densely populated areas. So much water goes through human use and is treated and released. That water can make up a huge percentage of the flow of the receiving systems. There are all these great charts on the web about human population and how we are all becoming more urban. The U.S. population is about 80 percent urban—defining urban broadly to include urban, suburban, etc., but 80 percent of us live in or near a major city. Worldwide it is over 50 percent now that live in urban areas. That means a lot of water is being used, treated and released. Even if our treatment plants function beautifully and take out all the pollutants and all the organic carbon, the water from that is not going to be the same as the water that would be in a river or stream naturally. It is going to have some kind of an effect. That gets into the issues of pollutants, but also just how the human footprint is affecting the ecosystem. These urban rivers—even if they are a natural occurring river—we are putting so much of our treated water in them that they are almost becoming built environments. In the Chicago River, for example, 70 percent of the water is effluent. It has been treated, but it is effluent. It is not natural water. And in DuPage County, even in the suburbs, some of those rivers are 30-40 percent effluent. That is a lot. I am not trying to say that treatment plants are bad or that we shouldn’t use water...
any more, of course. There are things we can do to improve treatment plants, and we should always try to improve them, but no matter what we do it will never be exactly the same. We have to understand how our activities are changing these ecosystems. That is something I am hoping to continue looking into.