UpClose

ISSUE 12

TIM HOELLEIN

From his lab at Loyola University Chicago, aquatic ecologist Tim Hoellein investigates the interactions between common pollutants and organisms in rivers and streams. After several years of investigating microplastics in waterways flowing from Lake Michigan, he has now turned his sights to measuring and beginning to document the impact of plastics carried into the lake by area rivers.

What first sparked your interest in plastic pollution?

It actually started while I was doing a different kind of research on nitrogen pollution. Wastewater and agricultural operations contribute a lot of excess nutrients to coastal environments, streams, and rivers. Those added nutrients cause extra algae to grow, some of which contain toxic chemicals. Then the algae rots and decomposes, and the low oxygen levels that process creates can kill off a lot of things. That is what most of my research has focused on: how organisms process that nitrogen, and what happens to the nitrogen along the way.

I was doing that nutrient pollution work in New York City, which was where I lived before coming to Loyola. We were working on oysters and oyster restoration. There is interest in bringing oysters back to New York Harbor in order to increase the number of filter-feeding organisms. Oysters may be able to eat some of the extra algae that's stimulated by wastewater and runoff. New York has the same wastewater system problems as Chicago: the sewage infrastructure really wasn't built to withstand all the wastewater we now have, although they've improved the infrastructure in a lot of ways.

So, we'd been working on all these oyster projects for a couple of years in New York—in the East River and some in the Hudson. While doing that, I noticed a ton of garbage along the shorelines. This is a really urban environment. Some of this stuff, I assume, is coming off the coast, some of it is coming down the river, and some comes just right from the city, so there is a ton of garbage along the

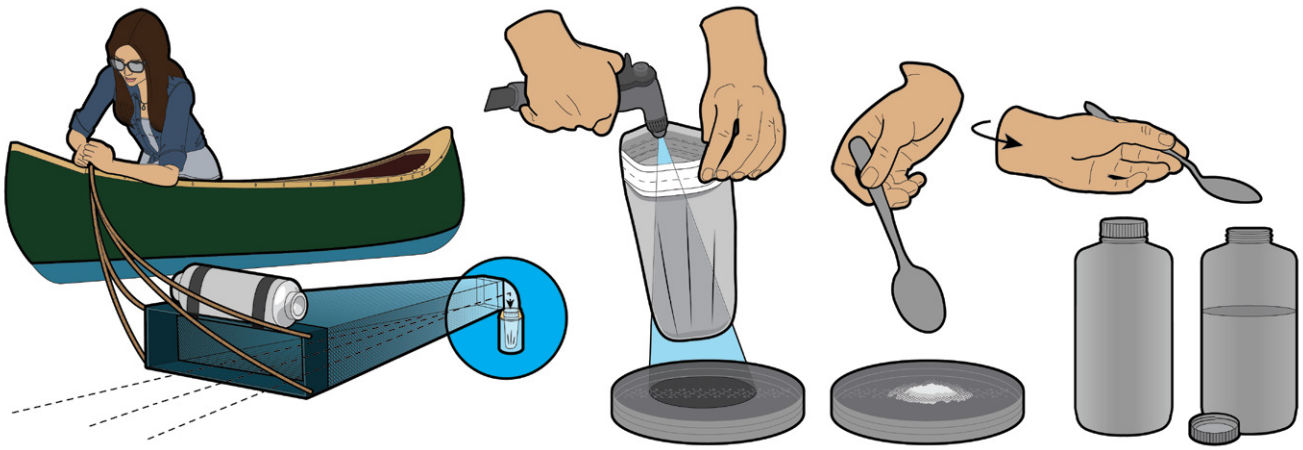
beaches. And yet, the water itself wasn't in that bad of shape.

We were working on this reef that the government had constructed at some expense—a couple of million dollars of oysters—in the East River in the Bronx, and next to this reef was a giant pile of tires people had thrown into the river. There were oysters all over the tires. My thought was, "Oh, here's a reef for essentially free." I don't know if you'd want to eat any of these oysters, but they were growing well on the garbage surface. This got me thinking about the ecology of litter and how it interacts with living things.

When I moved to Loyola, we were doing similar projects on invasive clams and mussels in the Chicago River. It was the same situation. We were studying these clams—how they process nitrogen and where the excess nitrogen goes. Then I noticed all this garbage all over the place and thought it was really similar to what I had seen in the East River and in marine environments. I knew litter was a topic people have studied largely in the open ocean, so I started reading the scientific literature thinking, "Maybe this has been studied in rivers as well." I discovered there hadn't been much research on this stuff in rivers. I realized we could start counting it, measuring it, and determining how the freshwater environment compares to the marine environment as it relates to garbage and litter. I started doing that, and then we published a few papers on garbage on Lake Michigan beaches and in the rivers around here. That's what lead me to the topic of microplastics.

SLOW AND STEADY

MICROPLASTIC SAMPLING IN RIVERS AND STREAMS



Researchers collect microplastics using a fine-mesh net deployed for 20-30 minutes at a time. The natural and man-made materials trapped in the net are then washed into a sieve and moved to a bottle to be transported to the lab.

When we're talking about garbage and litter, we're not just talking about the stuff you see but also these small pieces of plastic that aren't visible and have their own unique toxic properties. I read about microplastics in the ocean and thought, "Maybe someone has mentioned it in rivers," but again it turned out that people hadn't really done that work. Then I thought, "I can do that." I came at microplastics from a different direction, but all of these urban pollution issues are happening at the same time. When you're working on one, you observe the others.

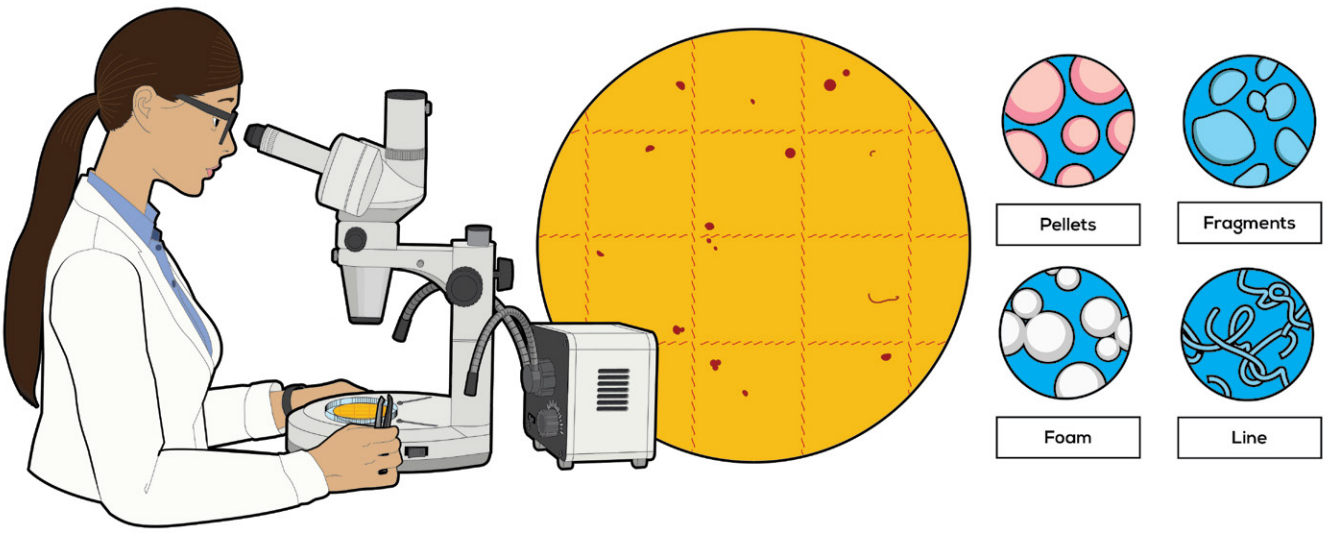
You've had a couple of projects focused on microplastics, including one where you found bacteria that cause gastrointestinal disease. Can you tell us about that project?

One of the things we've learned about microplastics is that wastewater treatment plants can be a source, which is surprising to people. These small plastic beads, spheres, and all kinds of other shapes are used as abrasive devices in toothpastes, soaps, and other consumer products—and then there's the plastic in the synthetic textiles we wear. These fibers break off in the washing machine, and all that goes down the drain to the wastewater treatment plant. Some of it comes out in the effluent and flows into Lake Michigan, the other Great Lakes, rivers, or wherever else the effluent discharges.

We wanted to really quantify if wastewater effluent was the point source of microplastics in rivers, so we went to about 10 rivers in Illinois and measured the concentration upstream and downstream from the treatment plant. This study was funded by the Illinois Water Resources Center. We found that, in most cases, there were higher concentrations of plastic downstream. The effluent was contributing microplastics to the rivers.

SMALLER THAN THE EYE CAN SEE

MICROPLASTIC SORTING AND COUNTING



Microplastics pulled from waterways are separated from organic material before being manually counted and sorted by pairs of graduate students working in tandem.

One of the associated biological implications was that there are, unsurprisingly, a lot of microbes typical of our gastrointestinal tract on the plastics from the wastewater. The system deals with human waste, so many of the microbes that live in wastewater treatment plants are indicative of gastrointestinal microbes. The longer those microbes are out of the gut and in environmental conditions, the more likely they are to die. They don't do well outside the human body. The wastewater treatment process is designed to lengthen the flow of the water through the plant in order to kill these microbes. Some plants use disinfection at the end—UV light or chlorine—to kill any remaining microbes. They do a great job trying to stop them from entering the environment. But we were still interested in what microplastics look like compared to other microbial habitats floating in the water or laying along the bottom of the river, so we analyzed the communities on the plastics downstream of the wastewater treatment plants. We found that the microbes on those plastics still looked a lot like the gastrointestinal microbes. Somehow, through a process we don't exactly understand, the plastics were allowing these gastrointestinal microbes to survive the treatment process, whereas they weren't persisting on the surface of the organic material in the water. That's not a process we fully understand, but we documented it at a number of sites.

Do you have a hypothesis for why that is?

Two of the most common microbe groups we found were *Pseudomonas* and *Campylobacter*. There has been some work on those organisms that suggests they like to be in a community together. So, there may be some sort of positive community mechanisms going on where they support each other in the way that biofilms grow on these plastics. That's kind of a shot in the dark supported by laboratory incubations of these organisms. We're not really sure. Maybe the plastics are somehow able to provide surfaces—nooks, crannies, or hiding spots—for some of these bacteria to avoid the disinfectant in a way that organic surfaces don't allow for. It's going to require some laboratory experiments to see what we can find out about the community and their susceptibility to different environmental conditions.


Biofilm

What could the presence of these bacteria mean for the river ecosystem?

We don't know. We're curious, though. We originally measured just downstream of the wastewater plant, so the next year we went back and also looked one and two kilometers downstream. This was in the North Shore Channel in Chicago, which has a big wastewater treatment plant that flows into it. It's a sewage canal with a little bit of Lake Michigan water. We wanted to see if the concentrations stayed the same as you move down the canal or if the microplastics settled out. We also wanted to see how the microbial community changes. We found that it didn't change that much. Over the two-kilometer distance, we saw the same amount of plastics in the water and the same community of microbes. We're going back to take measurements 20 kilometers downstream to see if the concentrations and microbial communities change. We anticipate that, just like in the wastewater treatment plant, the longer the plastics are in the environment, the fewer gastrointestinal microbes will persist.

Were you surprised the microbes made it the two kilometers?

Yes, we were. And we don't know what to make of that exactly. These plastics could be a novel way of distributing these organisms that didn't exist previously. If they come into contact with fish, birds, or people—that could be something we don't want. It'd be good to know what kind of community is out there and how much of the community is out there. We don't really understand that yet.

What happens after you take the samples? What does the bacteria analysis look like?

We do two things. We want to know how many plastics are there, and we also want to know what microbes are on the plastics. Those are two different measurement processes.

We start with the microbial communities right away. We bring back the samples, pick out the plastics while leaving the microbial community intact, put the microbes in a vial, and freeze them. They go in a -80˚C freezer until we can get to them. Those vials represent what was in the river. When we come back later, we'll do gene analysis based on the 16S rRNA gene. That's a very common, widespread way of measuring microbial communities and identifying each member. It's a particular gene that all microbes have. We can extract the DNA from the sample and amplify the gene. That gives us a sequence, and then we can see what the community of microorganisms is.

To measure the plastics, we run them through a digestion process to get rid of all the organic stuff. Then we put them on a filter and count them.

Does the gene analysis tell you the family or the species?

You can get down to the genus. That's useful, but one of the problems is there are hundreds of thousands of microorganisms on there, so many of them come back as unidentified bacteria. There is a lot to learn in the field of microbiology in terms of what's out there and how we use this particular gene to analyze communities. It's useful for determining what family or genus the bacteria are in, but it's not useful for determining what the bacteria are actually doing. There are a lot of other genes that give them different types of metabolisms. Those genes tell you more about what the bacteria are capable of doing—breaking down this sugar or that protein, carrying out photosynthesis, or maybe being resistant to antibiotics. But this particular gene just tells you what family they fall in.

Our hope is to go to that next level and sequence the entire genome of every microbe in a sample using a new technology called metagenomics. It's really expensive. It's a couple thousand dollars a sample. That's not doable for us yet, but the prices are always coming down. Hopefully we can get to the point that we'll be able to see not only what the makeup of the communities are but also what they do. That would be really good to know.

Since the microbes are frozen, can you wait until the technology is cheaper?

Yes, exactly. We keep all our samples. In the North Shore Channel, which is our closest study site—we've been going out there for three years taking samples upstream and downstream of the wastewater treatment plant and looking at the plastics.

Until recently, Chicago was one of the only places in the country that did not use any disinfection—that was kind of notorious. They started disinfecting in April at the plant we're looking at on the north side. We have samples going back two years before the disinfection. And now that they've started, we can get our "after" samples and see whether disinfection does help reduce some of these potentially dangerous microbes on the plastics. We also went to one of the smaller wastewater treatment plants in Chicago called Kirie Wastewater Reclamation Plant. We went there before they started their disinfection and after, so we will have that comparison as well as the North Shore Channel comparison.

How small are the pieces you're collecting?

The plastics we're looking at range from about 0.3 to 5 millimeters, and most are on the smaller end of that range. I know that's hard to envision. You can see about a millimeter. It's a little harder to see this stuff, especially when they have microorganisms on them because they just look like dirt. It isn't always obvious it's plastic. When you look at them under the microscope, they look like little threads, jagged fragments, or sometimes spheres. A lot of the time what tips us off is the color. They're synthetic looking. There are all these different colors—red, green, and blue—that aren't naturally occurring.

How do you collect, count, and categorize things that small?

We tie up a net that's floating about one or two feet below the surface of the water. We let that sit there for 20 or 30 minutes while we measure the flow of the water so we know how much water is moving through it. When we pull it out, there's this green goopy brown mess at the end of the net. That's gunk that's just floating down the river. A lot of the time, you can't see any plastics. We put all that in a bottle so we can process it back at the lab.

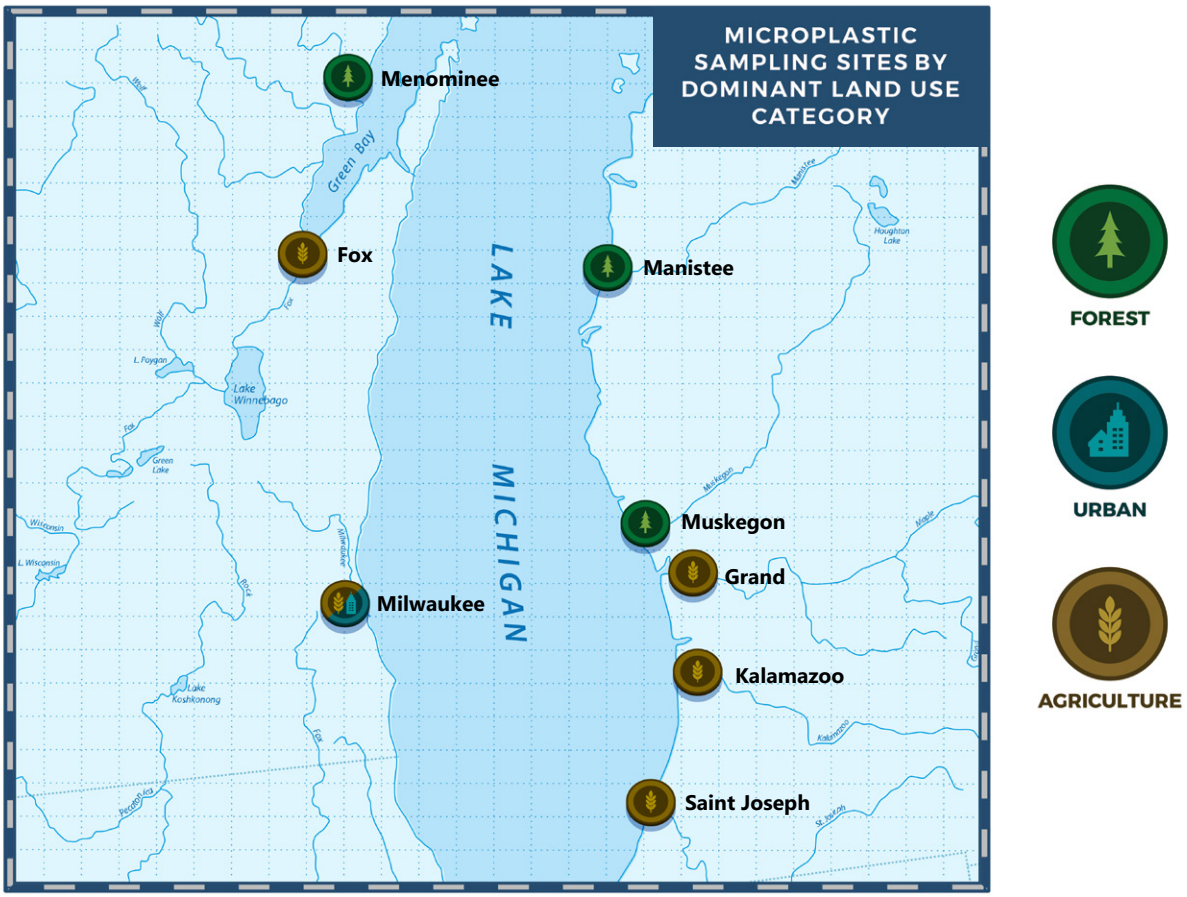
It takes a long time to process these samples. It requires first getting rid of all that organic material: algae, suspended soil, and other stuff that obscures your view. We use peroxide oxidation. This process uses hydrogen peroxide—similar to what you would put on a cut, but so concentrated that you wouldn't want to. It bubbles and fizzes and breaks down a lot of that organic stuff. But plastic is resistant to peroxide oxidation, which is why hydrogen peroxide can be stored in plastic bottles. After that, we do a salinity separation where we add as much salt as possible to the water. That changes the density of the water. We get that into a beaker and pour it into a funnel. Any sand or other heavy stuff will sink to the

bottom, and the plastics will float to the surface. We just let it sit overnight to separate. Then we discard the junk at the bottom, and the stuff at the top gets moved to a filter with a gridded surface.

We manually count what's on the filter. Graduate and undergrad students work in pairs sitting next to each other at the microscopes. They each look at the filter, which is divided into quadrants and then individual squares, and count everything they see and categorize it as a fiber, fragment, or pellet. Then they switch seats and count each other's filter. Every filter gets counted twice as a quality control. If they have a question about how to categorize something, they'll both look and make a decision together. If we can't tell whether it's plastic or not, we won't count it. We try to be as conservative as possible in our counting. It's a lot of repetition. Science is not always exciting. There are a few days and nights out in the field and then a year in the lab. A lot of ecological research is like that.

You've just started a new project focused on Lake Michigan. What is the goal of that project?

This is a much larger scale project where we're trying to see how much rivers contribute to the plastic load in Lake Michigan. We'll be going to the largest tributaries to Lake Michigan—right where they meet the lake—and measuring how many plastics are in the water and sediment at that location to try to get an



idea of where the plastics in the lake are coming from. We'll be sampling three times over the course of a year—spring, summer, and fall—at the eight biggest rivers around the lake to try to get an annual estimate of how many plastics are coming off the landscape.

Why did you choose those tributaries?

We wanted the biggest ones, but we also wanted to get a gradient of urban land use. We want to sample the largest most urban and least urban tributaries. Our hypothesis is that the more urban locations are going to contribute more plastics from a lot of different sources, including wastewater. And we expect the more rural watersheds will have fewer plastics in them.

We're at the very beginning of this project. We've done a few sites to represent summer, and we'll sample again in the fall and in the spring of the coming year. Then we have a second year in the project.

Why do we need to know where the plastics in the lake come from?

In order to make priorities about management. There's a medical analogy here: you want to know the origins of problems so you can most effectively maximize your solutions or the time spent looking for solutions. If we don't know where the plastics are coming from, we don't know where to start addressing the problem or what to do to try and prevent it. There may be some surprises, too. It could be that there are other sources we are not thinking of. If we don't analyze it on a large geographic scale like this, we won't really know.

The same is true for the garbage we saw in the river—the big stuff. We try to measure and categorize it so that we can figure out where it's coming from. What's the most important source? What do we do to try to mitigate or prevent the problem? That's where the research is at now. We're at the very earliest stage, which asks, "What's the problem, what's the scale of the problem, and where is it worse?"

What will happen in year two of the project?

We'll pick a few tributaries—I'm thinking maybe two depending how intensely we want to sample—and sample along the whole length of the river to try and get a longitudinal assessment of the microplastic concentrations and associated microbial communities. What we want to know is whether there are landscape features or different components of the river that might increase or decrease plastic concentrations. For example, we think the dams situated along some rivers' length may cause the plastics to settle out and decrease concentrations. But we don't know. We won't know until we measure upstream and downstream of dams, so we have to go and do that. Even things like big bends in the river could slow down water and promote deposition.

People have been studying rivers and how they move natural materials like soil and water for a long time. There's a lot of literature out there about how particles move through rivers and end up at the ocean. We have a lot of hypotheses based on natural materials that are the same size as microplastics, but those have not yet been applied to plastics. We're going to try to do that.

Will the longitudinal surveys span multiple seasons as well?

I think we'll be able to do that work just over the summer. It depends on how many samples we get. The volume of samples gets high very fast. We have to be as strategic as we can to maximize the number of samples and still be able to accomplish things within the timeframe. This is always how it goes for any site. You want to sample as much as you can, but you also want to be realistic about what you're going to learn and when it's going to get done. We'll have a better sense for this during this current year. We're generating a lot of samples, and hopefully we'll be able to bring on a lot of students and get things processed.

When you're sampling across seasons, do you try to sample on days that are representative of that season, or do you look for similar characteristics across seasons?

We want something that's kind of representative of the season. We do the best we can, but there are also staffing issues to consider. We try to find a day when everyone is available and the conditions are right.

At the very least, we try to not go out under storm conditions. Part of that's for the safety of our researchers. At this point, there have been no studies that examine what we call baseflow, or low flow, conditions versus storm flow conditions. It just hasn't been done. These are probably different enough conditions to affect plastic concentrations, but we don't know. For this project, we're essentially doing a baseline analysis for non-storm conditions in each season to see what the plastic concentrations look like.

How do you know what's representative for the river and season?

All these rivers have gauges maintained by the U.S. Geological Survey (USGS), which has river gauges throughout the country. It's nice because the results are all online. USGS has a great website that is divided by state where you can go and look at river discharges.

Are you able to determine the source and age of the plastics you collect?

That's hard to do. For source, we essentially just infer based upon the shapes. The spheres, for example, give us some indication of potential wastewater input. The same with fibers. We anticipate that those are largely from either municipal wastewater or industrial wastewater that's entering river. The fragments we see—which are these amorphous jagged shapes—we think those may be from larger pieces of plastic that have been broken down over time.

We don't know of any way to determine the age. Marine biologists have struggled with this too. We just try to do the best we can based on the shapes and what we know about the sources of those shapes.

The smaller it is, the older it is?

Potentially. It depends on how the plastic was broken down.

Plastic doesn't break down easily, does it?

Biological breakdown is super slow. It takes a very, very long time. But there are physical processes that can break down plastic particles. Freezing and thawing can break them apart. Wetting and drying—you know, sometimes the materials get stranded on the riparian area or the streamside area. It's dry, and then it's wet, and then it's dry again, and that can break down plastic. Currents, wind, and sunlight can also break things down. It's largely these physical processes that we think are breaking things down, along with a very small amount of biological activity. There are some microbes that can break plastic polymers apart.

Do we know how quickly these processes break down plastic?

No. People are trying to do that work, but the breakdown is really slow, so it's hard to document it exactly. I know there's a group in Canada that puts plastic out on beaches and measures its loss of strength—the material becomes more brittle sometimes. Or they can take electron microscope pictures to look at scratching at a very small scale. That's kind of the best data we have.

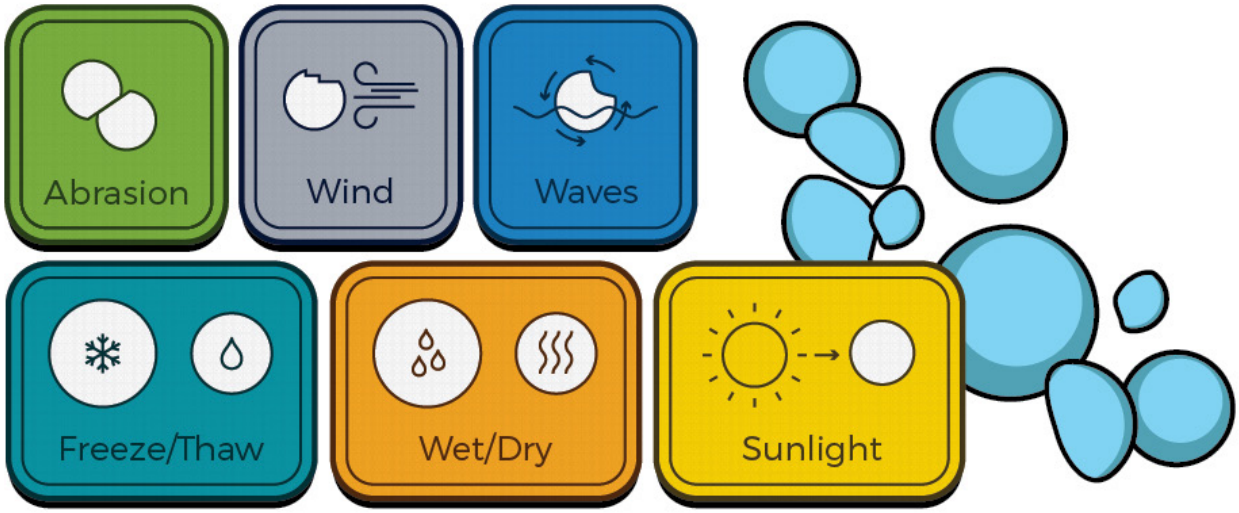
Your Lake Michigan project will also examine the role of microplastics in the ecosystem, correct?

Again, we're at an earlier stage, so we're still just trying to figure out if it's in aquatic wildlife. We know plastics are colonized by microbes and other organisms in the river to the point that it doesn't look like plastic. At that point, it becomes a potential food source for fish and other animals. We're basing this on marine research that has shown that plastics are consumed by just about everything. Filter feeders, like mussels and oysters, and also crabs and fish—we find plastics in all these organisms. We assume this probably also happens in freshwater. If the plastics are there, they're probably eating them. But this hasn't really been well documented in freshwater systems.

We're collecting bugs and fish at these eight rivers and freezing them. The bugs—I think we'll just throw those in the peroxide whole. For the fish, we'll cut out the digestive system and put that through the digestion process with the peroxide to see if there are any plastics there. So, we process it just like we do our net samples except we're looking at the tissues in this case.

THE LONG GOODBYE

BREAKING DOWN PLASTICS



It takes hundreds of years for bacteria and other living organisms to biodegrade plastic. Some physical processes can speed up degredation, but the breakdown is still so slow that it's actually difficult to measure.

We're doing this work now. We have done the St. Joseph and Milwaukee rivers at this point. We analyzed one fish from Milwaukee Harbor already and found that there were a lot of these plastic fibers in the gut.

At this point, our wildlife assessment is really just presence versus absence. We're not examining how plastics affect their health or if they get stuck in their tissues. We just want to know if they're there. People working in the oceans are always several steps ahead of us. They have been doing work like feeding organisms plastics to see if they get stuck in their tissues and what kinds get stuck. There are also chemicals that stick to plastics or are part of the plastic itself that leach out. And when they're in the digestive system, which has no oxygen, the leaching can be rapid. They can see if the chemicals come off the plastics while in digestive systems. One study looked at crabs that eat mussels with plastics in them, and they found that when the crabs ate the mussels, the plastics got into the crabs and stayed in there—the crabs didn't pass them—so there's potential for bioaccumulation, like what happens with mercury or PCBs. There are still a lot of open questions about whether that happens. But in the Great Lakes, we have virtually none of this data yet. We're just seeing if the plastics are there. That's where you have to start, I think.

Are you testing all the fish you catch or targeting a specific species?

We would like to be that sophisticated, but we're doing more elementary minnow traps and seining. We use these nets where two people can walk along each side of the river and trap small stuff. We're looking at mostly small fish that are right where we put our little canoe. We're looking at the base of the food web. It would be nice to know what is in sport fish, but I don't think that would be included in our study at this point.

Are the species similar enough that you can compare findings across rivers?

I don't know the answer to that yet. I was looking for information about what fish live in these harbors, but I couldn't find anything. So I thought, "We'll just measure what's there." I imagine we'll find the same species. There are not a ton of species in the lake. We found a lot of fish in Milwaukee Harbor, and I think that site is in the worst shape environmentally. If they're there, they are probably in other places.

But the other way we can group these organisms together is by what they call functional feeding groups—the way they eat. Some species have sucker mouths, so they root along the bottom, whereas some are more predatory. Even if we don't have the same species, we could have the same groups, the same feeding strategies.

Will you also sample fish during the longitudinal survey in year two?

I'd like to, but I don't know yet. That's a lot of samples. It depends on how big things get this initial year with the fish component. When we're sampling in the river, we don't always have a spot shallow enough that we can collect these minnows. I don't know if it's going to be possible. We'll see what we end up with.

You piloted different methods to test for the presence of plastics in organisms. What were you hoping to learn?

We wanted to find out what works best for getting rid of tissue and leaving plastic behind. People have done this removal in a couple of different ways with different chemicals. We have stuck with the peroxide digestion for our net samples, but with fish or other organism tissues, people have used an acid solution to try to digest the tissues. What we wanted is to see if there's anything left after you set a fish stomach in acid overnight. That's kind of it. We were just interested in what's going to work best for clearing out the organic material based upon what other people have done.

It turns out concentrated peroxide works for us, so we've stuck with that. It seems to work pretty well on tissues that aren't very fatty, which is what I'm gathering. If we were doing tests on organisms that have more fat in them, like tuna, we would have to use the acid approach. The peroxide isn't good with fatty tissues. But we're working with these little minnows, and their guts are not that big or fatty. We tried the acid, we tried the peroxide, and we've stuck with the peroxide.

What questions related to plastics and litter do you hope to tackle in the future?

I have another grant from the National Science Foundation (NSF) to continue working on the larger pieces of garbage we've been measuring, as well as microplastics, and examine how they influence ecosystems. One of the things we're going to be doing next on microplastics, which overlaps with that NSF grant and our project with Illinois-Indiana Sea Grant, is to try to measure deposition rates, or how fast the plastics settle out of the water onto the bottom of the stream. Most deposition work done for organic particles is measured by releasing and tracing corn pollen or something else easily identifiable to see how it flows through a river. You can't do that with plastics because you don't want to release plastic into a river. It can be challenging to get this type of data. We have to resort to using artificial streams.

We want to know how the plastics move, how much stays in the river, and how that amount is affected when the pieces are colonized by microorganisms. We think biofilms are going to make the plastics stick a lot more to different surfaces in the river. There's a whole system of measurements you can do when you put this stuff in the river. And you can actually extrapolate to bigger rivers and say, "Here's our estimate for how much is retained in this stretch of the river and how much is going downstream based on real data measurements taken in streams." That's a lot of what people want to know—what I want to know. We know that rivers discharge plastics to the ocean, but we don't know how much goes in and stays there. That's totally unknown, and I think it's possibly a pretty high number. Doing these kind of tracking measurements can help us get there. We want to take some of the fine particle or organic matter predictions and apply them to microplastics in order to get a number for how much is retained.

How long is the NSF-funded study?

Five years. It just started in July. It's our first project related to microplastics that's trying to really quantify deposition and retention.

Your work involves a lot of students. How do you choose students for your lab?

The way I pick them is usually based on their interests. We have a lot of students here who want research experience. Many of them are pre-med. We have a big biology program here with mostly pre-med students, and many of them want research experience even if it's not directly related to medicine. We have a smaller contingent of biology majors who are interested in ecology and the environment. I try to give preference to those students because a lot of our department services the pre-med students, and there are a lot of other people who do research in genetics or cell biology that would be more directly applicable to their medical school applications. I try to find the ecology students. That doesn't always work, and I've had some great pre-med students too.

With this kind of work, you have to sit at the microscope for a couple of hours at a time. It doesn't really work to come in for 30 minutes or an hour. So, we try to figure out if this is going to work for their schedule. Then I try to reorient their expectations of what ecological research means, especially as it relates to laboratory work. Some students find out it's not for them. That's okay. It's a learning process for them.

How have these experiences influenced their careers?

I've had a few go on to ecology programs in graduate school, and a few more are interested in doing so. A couple of my students have gone on to master's and PhD programs and are doing research. None of them have been interested in studying garbage so far, but they've taken their experience from our field and lab work and applied it to other things. For example, one is studying lakes in Iceland for a study on global warming and insects. Another is working on invasive species in lakes at the University of Minnesota. They've gone into more community biology rather than litter. But they apply a lot of the same techniques that we use for analyzing data sets in that work. I've been really pleased.

Illinois Indiana Sea Grant
Illinois Water Resources Center
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