By
Michael A. Kallok

Diving for Aliens

A little-studied group of animals is getting a closer look in Minnesota

John Leopold and Karl Anderson, a researcher at the University of Minnesota Crookston, dive for freshwater sponges as part of the state's first comprehensive study of these obscure organisms.

Photography
by Steve Philbrook

On a sunny August day,

I'm wading in the Mississippi River near Bemidji looking for a bright green animal that very few people know much about. Karl Anderson, a researcher at the University of Minnesota Crookston, knows more about them than most. He finds one attached to a rock beneath the shadow of Power Dam Road Bridge.

To me it appears like an amorphous clump of algae slightly smaller than a tennis ball. But as I move in for a closer look, I can see its asymmetrical form beneath the moving water. Upon Anderson's suggestion, I squeeze one of its armlike projections. It is more rigid than I expect and has a slightly rough texture as I compress it between my thumb and index finger. I'm surprised by the way it springs back to its original shape when I let go.

"It is a sponge," I conclude. This elicits an affable chuckle and confirmation from Anderson, who is working on the most comprehensive investigation of freshwater sponges ever conducted in the state. While freshwater sponges have been known to science for a long time, they have rarely been studied. Anderson is here to develop a protocol for gathering, preserving, and analyzing sponges ahead of the study.

Sponges may get overlooked because they are considered the simplest of all animals, says Anderson. "In the evolutionary scheme of things, everything kind of goes off in one direction and has more developed tissues," he adds. Sponges are immobile creatures that have not developed true tissues—

Karl Anderson searches for freshwater sponges in the Mississippi River near its headwaters.

think muscles, nerves, and skin—that he says "are needed to evolve into something we want to think of as greater."

That doesn't mean studying freshwater sponges is simple. Before the advent of the electron microscope, it was impossible to conclusively distinguish one species of sponge from another. Color, shape, and size offer few clues to the taxonomy of freshwater sponges, which are abstract masses of simple cells held together by tiny needle-shaped silica bodies known as spicules. Microscopic features on these spicules and reproductive bodies known as gemmules are one key to identification. Advances in genetic analysis provide another.

University of Minnesota Crookston researchers will use both identification tools as they explore the diversity and distribution of sponge species in all 10 of Minnesota's major watersheds. This three-year study, which was made possible by a $258,000 grant from the Environment and Natural Resources Trust Fund, will also examine the ecological role of freshwater sponges and what their presence means for water quality.

Comparing Notes

After our stop in Bemidji, Anderson and I head to Itasca State Park. At the Gulsvig Landing near the headwaters of the Mississippi River, he and biology technician Michael Laurich pinch gumdrop-size chunks from several sponges. They transfer each sample into a small vial and label it. Then they record location, water temperature, and depth, measure the amount of dissolved oxygen in the river, and gather a water sample.

Not far from here, the last substantial research into Minnesota's freshwater sponges was conducted more than 40 years ago. In the summer of 1970 Louise Rollins-Smith, then a University of Minnesota zoology graduate student looking for a Ph.D. project, began collecting sponges from Lund's Pond, a shallow lake in Itasca State Park. At the time, scanning electron microscopy was a relatively new technology, says Rollins-Smith, who remembers being captivated by the large, three-dimensional images of spicules revealed by the high-powered microscopes.

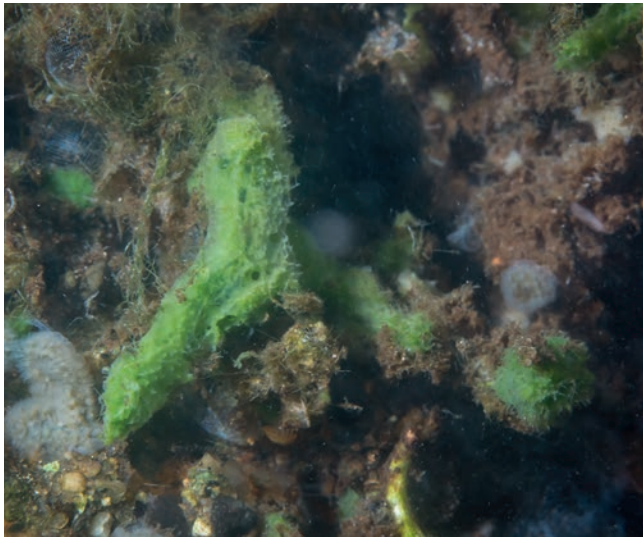
"They are just gorgeous," she says of the fine details of spicules and gemmules. Under powerful magnification, spicules feature intricate hooks and spikes. Gemmules have diverse features ranging from small cog-like projections to ones that resemble miniature umbrellas. By comparing her microscopy images with sketches of spicules made by biologists who had previously documented sponges in the upper Midwest, Rollins-Smith was able to positively identify five species of sponges in Lund's Pond.

"I think it's a wonderful project," says Rollins-Smith of the University of Minnesota Crookston's sponge study. "Are there more? Are there a bunch more?"

Roughly 200 species of freshwater sponges have been identified worldwide, says Anthony Schroeder, who is leading the genetic analysis of sponges collected during the study. In North America about 32 species have been identified, according to Schroeder, who anticipates genetic analysis could reveal new sponge species in Minnesota. But there is another reason to believe the current study may turn up more.

Details Revealed

Under the magnification of a scanning electron microscope, minute features of spicules and gemmules can be seen. These images were created by Louise Rollins-Smith and Lynn C. Hyland from sponges collected in Itasca State Park during the summer of 1970. A feature on a Trochospongilla pennsylvanica *gemmule (top left) is shaped like an umbrella. The gemmule of* Ephydatia mulleri *(top right) has features shaped like two cogs on an axle. Higher magnification of the gemmule of this species (above left) shows additional cog-like features. The spicules of* Trochospongilla pennsylvanica *(above right) have curved projections, which appear only as small bumps under the magnification of a light microscope.*



Karl Anderson (left) collects a sample of a small white sponge growing on the stern of a sunken boat in Becker County's Pickerel Lake. Algae is responsible for the green color of this sponge (above), which was growing in the shallows of the Mississippi River. In return for a place to live, algae help provide food and oxygen for sponges.

Most freshwater sponge research in North America has focused on species that are easily found in the shallows of lakes and rivers, says Schroeder. "We want to dive in lakes," he says. "We want to cast a broader net to search for where they could potentially be."

Searching the Depths

The next morning, I meet Anderson at Pickerel Lake in Becker County. We're joined at the public access by local scuba diver John Leopold. He knows his way around the lake, which is popular with local divers because of its clarity. Sponges will grow on any hard surface, and our plan is to search a communications cable and a sunken boat for specimens.

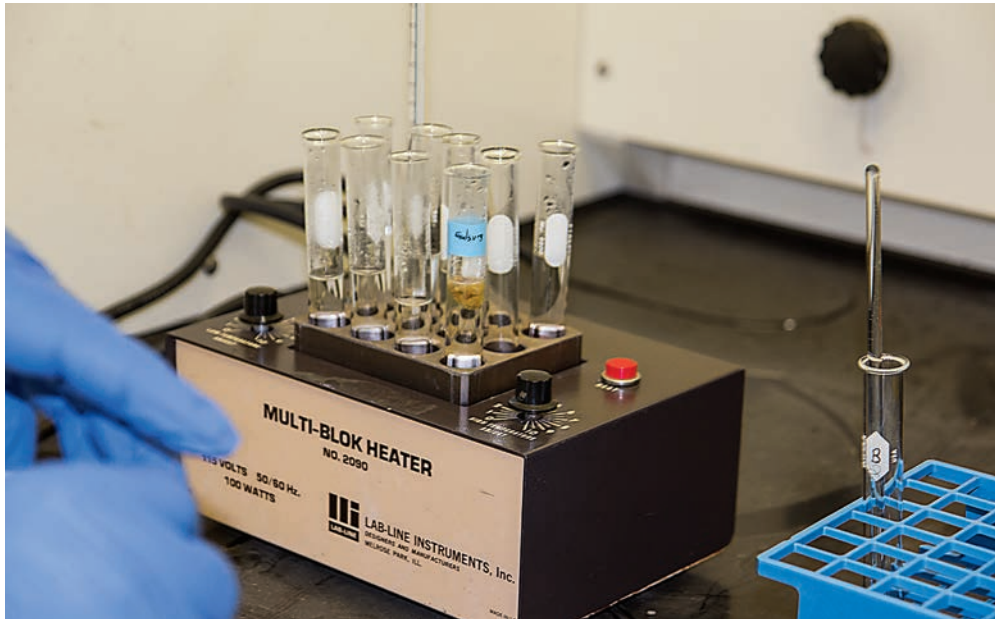
Upon our descent, we're greeted by a school of large bluegills. The fish, Leopold has jokingly warned, are accustomed to handouts from divers and may be aggressive. The bluegills circle in front of us insistently as we swim, but these escorts give up before we reach the communications cable a hundred yards from the access. In about 15 feet of water, Anderson points out a wispy, bone-white sponge no larger than a pushpin growing on the 2-inch-diameter cable. It's too small to sample, and we don't find any others, so we set a bearing for the sunken boat.

Unlike marine sponges, which grow year-round in the relatively stable environment of the ocean, freshwater sponges must withstand cold, darkness, and occasionally drought. To survive these events, freshwater sponges develop specialized clusters of embryonic cells called gemmules. As the sponges die back in winter, these asexually produced poppy-seed-size gemmules drop to the bottom of the lake or river. When conditions are favorable again, a new sponge will grow from the tiny armor-coated survival pods.

Rains the night before had clouded the water of Pickerel Lake, but the eight feet of visibility was sufficient for Leopold to locate the boat. The 1960s fiberglass runabout sits in 20 feet of water and has become an attraction for divers. A northern pike is using the boat for ambush cover, but it slinks off as we approach.

Anderson searches the boat and finds a specimen about the size of a golf ball growing on the stern. He transfers a sample into a vial, tucks it into a pocket, and we follow Leopold back to the access.

Unlike the bright-green sponges we found in the shallows of the Mississippi



Nitric acid (top left) is poured into test tubes containing sponge samples. When it is heated under a fume hood (center), the acid dissolves organic material but not the silica-based spicules and gemmules. Technician Michael Laurich (right) uses a centrifuge to concentrate the skeletal remains of the sponge samples.

River, the sponge growing deep in Pickerel Lake is white. It's possible that it's a different species, but Anderson won't know until the spicule and genetic analysis is done. It's also possible that it was growing in water too deep for the symbiotic algae that's responsible for the color of many freshwater sponges.

Indicator Species

All sponges belong to the phylum Porifera, meaning pore bearer. Sponges feed by filtering a constant flow of water through a network of pores. As they draw in and consume tiny organic particles, they also accumulate contaminants.

Because sponges are eaten by aquatic invertebrates such as caddisflies, which are preyed upon by fish, the study will also examine whether sponges are facilitating the transfer of pollutants up the food chain.

Venugopal Mukku, who worked to secure grant funding for the sponge research, will be leading chemical analysis of collected samples. It's known that marine sponges accumulate pollutants such as polyaromatic hydrocarbons, says Mukku, whose doctoral work explored the chemical composition of sponges in the Indian Ocean.

Mukku and associate professor Timothy Dudley will be looking for hazardous organic compounds in freshwater sponges as well as other contaminants such as antibiotics. They will also be testing water samples taken at each location where sponges are gathered. That analysis will try to find correlations between sponge growth and the presence of pollutants such as phosphates, nitrates, and metals.

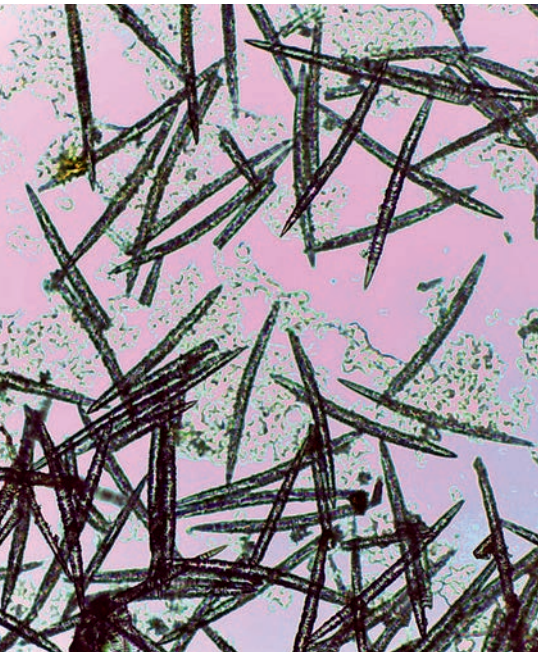
"One of the big things with this project is trying to get a good sense of where freshwater sponges are and what things are influencing that," says Schroeder, who notes that much of the scientific literature suggests freshwater sponges tend to be found in pristine areas. "They're good indicators of water quality."

If we can better understand the conditions favorable to sponge growth, we may be able to use their presence as a benchmark for water quality improvements, says Schroeder.

Discoveries Await

Under a fume hood at the University of Minnesota's Itasca Biological Station, technician Michael Laurich places a portion of each sponge sample in test tubes of nitric acid and heats it to 100 degrees. The process will remove any organic material and leave behind only spicules and gemmules. Anderson divvies up the remaining portions of the sponges for later genetic and chemical analysis.

Twenty minutes later Laurich transfers the tubes of nitric acid into a centrifuge and spins the samples at 3,000 revolutions per minute. When he pulls them out, a quarter inch of spicules, which look like fine white sand grains, have been pushed to the bottom of the glassware. He carefully tips out the nitric acid and rinses the spicules with water, then scoops some of them onto a slide.



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Michael Laurich (top left) searches a sponge sample for gemmules. Under magnification, spicules (bottom left) appear needle-like. A closer examination of these spicules along with genetic analysis allowed researchers to identify the sponge (opposite page) as Spongilla lacustris.

Under magnification of a light microscope, the spicules look like a jumble of suture needles. Laurich scans the slide for a gemmule and finds one. It dwarfs the tiny spicules. Laurich labels the slide and repeats the process for the rest of the samples.

During the winter of 2017, Anderson's spicule analysis and Schroeder's genetic analysis revealed two species in the preliminary samples gathered last summer: *Spongilla lacustris* and *Ephydatia mulleri*. Both of these were described by Rollins-Smith in a paper she published in the 1970s.

But one sample, the one collected from the boat in Pickerel Lake, has yet to be positively identified. Anderson is looking forward to the upcoming field season, when more discoveries will await researchers and their student assistants.

The project will also provide fertile ground for future research projects on sponges. "They are considered the simplest of animals, despite the fact that they aren't that simple," says Schroeder. "There are a lot of basic questions about the biology of these things that we just don't know, which is by itself fascinating."

Editor's note: University of Minnesota Crookston encourages citizen-scientists to report the location of sponges. Email umcspongersrch@crk.umn.edu *or call 218-281-8240.*