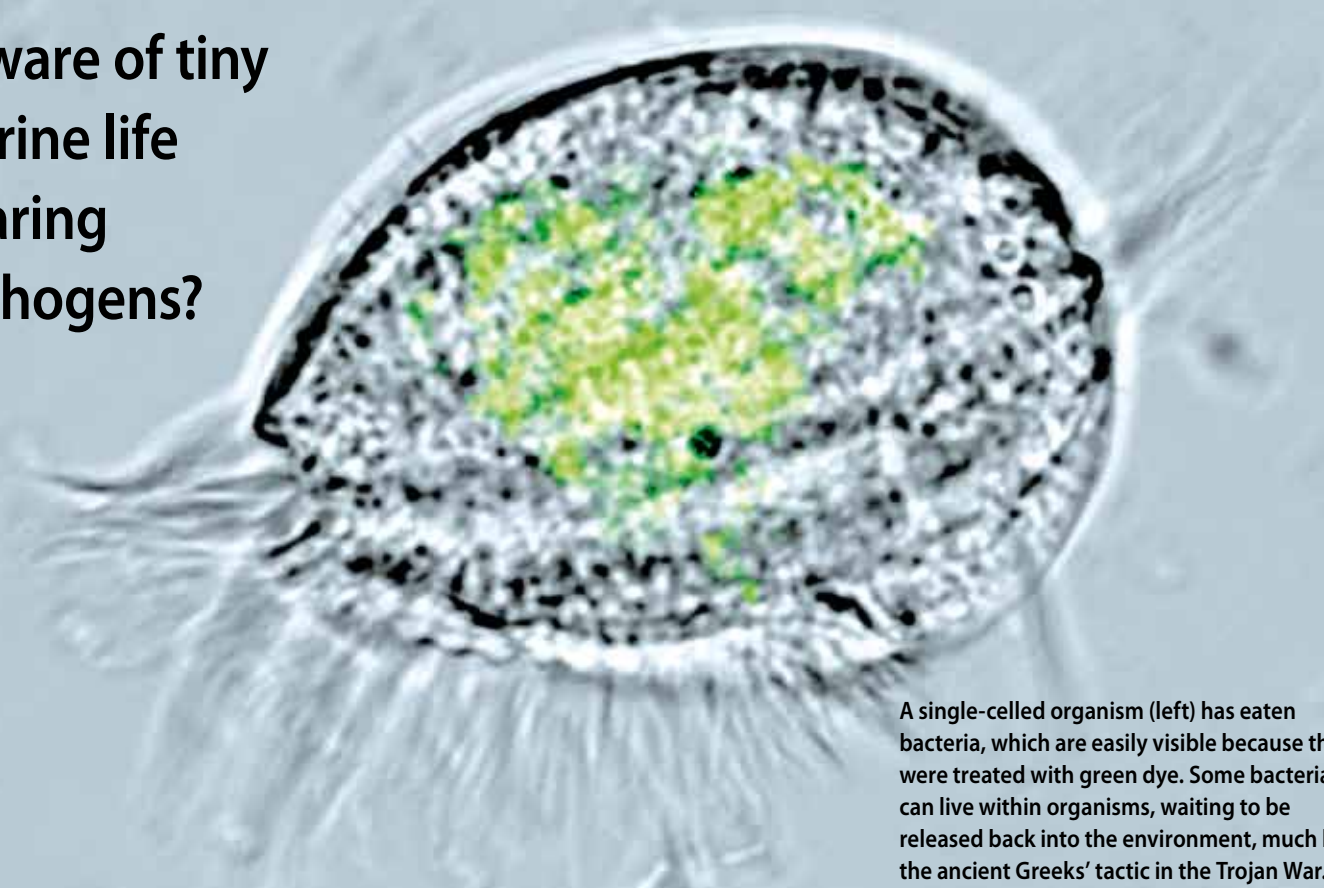


The hunt for microbial ‘Trojan horses’

Should we
beware of tiny
marine life
bearing
pathogens?



A single-celled organism (left) has eaten bacteria, which are easily visible because they were treated with green dye. Some bacteria can live within organisms, waiting to be released back into the environment, much like the ancient Greeks' tactic in the Trojan War.

In summer, Wood Neck Beach in Woods Hole, Mass., teems with tourists, but only a few seagulls kept Matt First company as he walked through the brown-gold marsh grass last November. Armed with a small red cooler containing sampling tubes, First was searching for Trojan horses.

No, these aren't ancient monumental structures hiding Greek warriors inside, but modern-day microscopic organisms in the ocean. Within these organisms, disease-causing bacteria may be lurking, protected and biding their time until they are ready to emerge and infect.

These microbial “Trojan horses” are single-celled organisms—amoebae and ciliates such as *Paramecium*, which used to be called protozoa, but are now referred to as protists. “These protists don't harm people, but they could contain pathogenic bac-



“Warren, come take a look at this virus I found in the patient's blood sample!”

teria, sometimes in very high concentrations,” said First, a postdoctoral researcher at Woods Hole Oceanographic Institution (WHOI).

“The Trojan horse idea is a relatively new field,” said Joan Bernhard, an associate scientist in the WHOI Geology and Geophysics Department and First's co-advisor. Scientists began using the term about 15 years ago to describe *Legionella pneumophila*, the bacteria eventually traced as the cause of the mysterious disease that broke out among people attending an American Legion convention in Philadelphia in 1976. It sickened 221 people and killed 34.

“Epidemiologists had a hard time finding the source of the disease,” First said. “They finally traced it back to water in air-conditioning tanks on the roof of the building where the Legionnaires were meeting,



Tom Kleindinst, WHOI

Matt First, a postdoctoral scientist at Woods Hole Oceanographic Institution, collects water samples from Wood Neck Beach in Falmouth, Mass., to search for microbial “Trojan horses” in the ocean. These are bacteria that can resist being digested by the single-celled organisms that eat them.

but they still had trouble finding *Legionella* within that water. It turns out that high concentrations of *Legionella* were living within the protists that lived in the tanks. *Legionella* were reproducing within these protists and coming out in small aggregates that became airborne in water droplets. Susceptible people breathed in high concentrations of *Legionella* that had just come out of their hosts and were looking for new hosts.”

“*Legionella* has given us the idea that this [relationship] may be possible in everything else,” said Sharon Berk, professor of biology at Tennessee Tech University, who studies *Legionella* and other protist-bacteria relationships.

Sanctuaries in the sea

Though confirmed in freshwater protists, microbial Trojan horses “have not

been studied very well in a marine habitat,” Bernhard said. “It’s very sketchy how prevalent they are in the ocean.”

That’s where First comes in. He is an early bioprospector, surveying what kinds of microbial Trojan horses exist in the ocean and investigating various biochemical strategies pathogens may be using to survive. His research also probes the question of whether an increase of untreated waste from sewers and large-scale animal farms is sending more pathogens into the sea and into these protecting protists, providing more opportunities for marine microbial Trojan horses to transmit diseases.

“Protists are abundant in nearly every aquatic environment on Earth, and they are the main consumers of bacteria in the environment,” First said. Thousands of different types of protists eat bacteria, he con-

tinued, “and they eat constantly. They can eat dozens to several hundred bacteria each hour. It’s been going on for 3 billion years, and it’s still going on constantly in every drop of seawater.

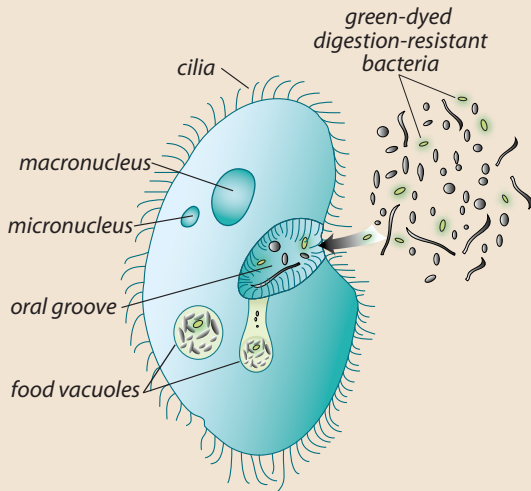
“But some bacteria have developed traits that allow them, when eaten by a protist, not to be digested, but instead to survive within that protist, to persist, to almost use that protist—its typical predator—as a host,” First said.

Within the protists, the bacteria find a safe refuge and protection from environmental dangers such as chlorination, or in some cases, antibiotics that would harm them. They also find plenty of simple sugars, made by the protists, that bacteria love to eat.

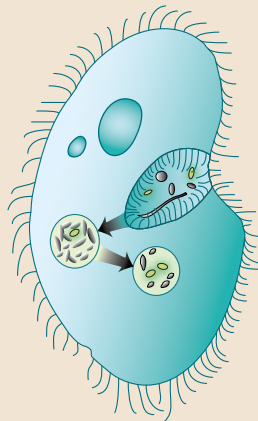
Being eaten without being eaten

At Wood Neck Beach, First knelt down

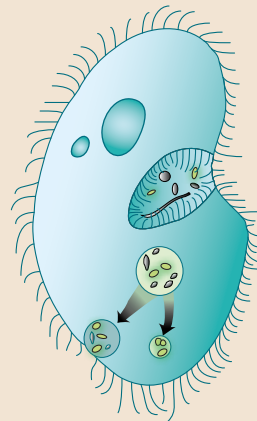
Ingested, but not digested



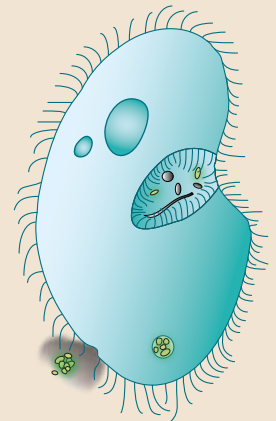
1 A unicellular organism, or protist, uses hairlike cilia to push assorted bacteria into its oral groove. Ingested bacteria are collected in food vacuoles—specialized organelles, or cellular compartments, where bacteria are exposed to acidic conditions and digestive enzymes.



2 Bacteria are killed and broken down in the food vacuoles, supplying protists with energy and nutrients. But some bacteria (stained green) can release molecules that interfere with the normal digestion process.



3 Digestion-resistant bacteria can survive within unicellular organisms or even reproduce within them.



4 Digestion-resistant bacteria, including some that can cause diseases in humans, can be released back into the environment.

at the marsh's edge to collect sediment using a core sampler, which works like a syringe. In every sample, there are tens of thousands of protists, including amoebae, ciliates, foraminiferans, flagellates, and dinoflagellates.

Back in the lab, First uses flow cytometry, a technique that passes water samples at

high speed through a laser. Analyzing how the laser light passes through cells in the samples, the flow cytometer can distinguish various cell characteristics, identifying about 1,000 cells per second and sorting out particular cells that researchers are interested in.

First began his research on the ciliate *Colpoda*, which has a healthy appetite for

bacteria. He fed the *Colpoda* bacteria called *Campylobacter jejuni*, which causes diarrhea in people. The bacteria were stained with a fluorescent green marker, so First could easily follow their journey through the *Colpoda*.

Bacteria eaten by protists are typically digested within 30 minutes. First measured the number of intracellular bacteria in ciliates for five hours, providing ample time for complete digestion. While nonpathogenic bacteria were completely digested in this time period, several undigested *Campylobacter* remained inside the ciliates, indicating the potential for a Trojan horse relationship.

First plans to continue testing other ocean protists and infectious bacteria with this technique, looking for other microbial Trojan horses in the sea.

Biomolecular subterfuge

First's work builds on the research of his co-advisors at WHOI, Bernhard and Rebecca Gast, an associate scientist in the WHOI Biology Department. Both study how bacteria can adapt genetically to harsh conditions.

Bernhard focuses on protists called foraminiferans and symbiotic bacteria that seem to help the protists live in extreme environ-



Scientists "tag" bacteria with a fluorescent green marker (in right tubes), which lets them see how bacteria are digested—or in some cases, not digested.

Tom Klemm, WHOI

ments such as the deep sea. Gast's lab works with amoebae and infectious bacteria. Her group has found *Legionella* harbored in several different types of marine amoebae.

Legionella has developed ways to protect itself from being digested, she said. Certain genes in the bacteria prevent the amoebae's lysosomes, a chamber full of digestive enzymes, from destroying the bacteria. The bacteria also hijack the protists' cellular machinery to grow. They use these same defensive tools to thrive in human macrophages—white blood cells in human tissue that engulf foreign bacteria as the immune system's first line of defense.

"Macrophages kill almost any other bacteria, but *Legionella* will replicate in macrophages," said Yousef Abu Kwaik, a professor of molecular pathogenesis at the University of Louisville, who studies bacteria and amoebae relationships.

Gast's findings raise questions: Could amoebae in the ocean offer an intracellular environment where bacteria can adapt and become human pathogens and/or develop resistance to antibiotics?

What's in there, out there?

In the age-old predator-prey relationship between protists and bacteria, First said, "we know that bacteria can adapt to the protists that eat them. They can change the size or surface of their cells to avoid predation, change the way they move, or dump out proteins and macromolecules that interfere with processes in the host."

To learn more about these survival strategies, First plans to analyze the genes of both the bacteria and the protists in the Trojan horses that he discovers. He also wants to develop his technique to measure what the protists release undigested back into the environment. Some bacteria may be able to pass through protists unscathed. Some of the fundamental biomolecular mechanisms he finds could one day lead to therapeutic strategies to counterattack bacteria and fight diseases in people.

First is not alone in his search for Trojan horses in the environment. Other researchers are looking for them on land. Berk's research, funded by the U.S. Department of Agriculture, is looking at whether ciliates

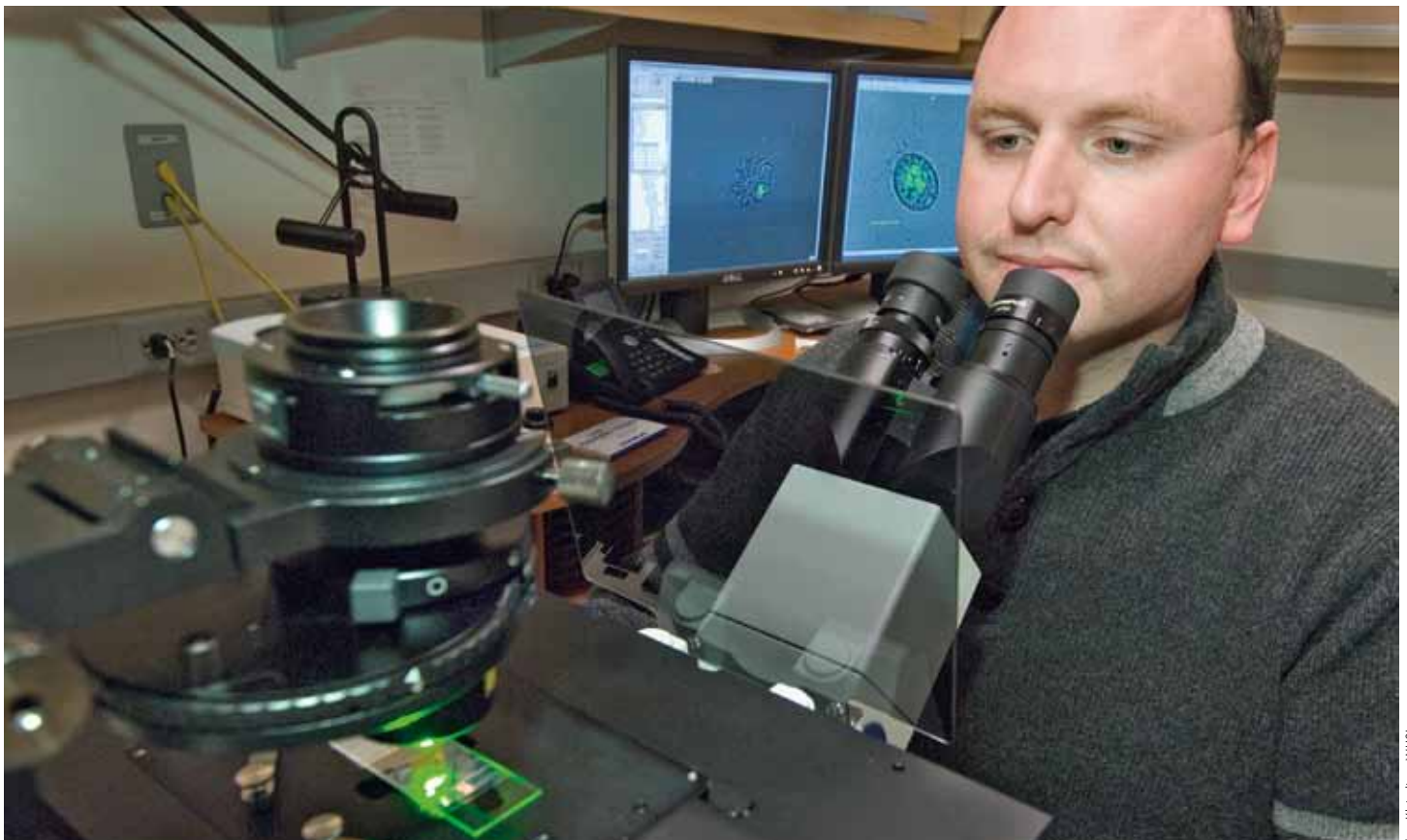
found on fresh produce may harbor infectious bacteria such as *E. coli* and *Salmonella*. Researchers have found *Salmonella* living in crop soil alongside protists such as ciliates.

The researchers are the first to admit that there is not enough evidence to implicate other Trojan horses for disease outbreaks in people. Other bacteria, such those that cause tuberculosis and cholera, can live in a protist benefactor, but it's unclear if the relationship plays a role in their infectivity. But scientists feel there is enough of a possibility to at least take a deeper look.

"We don't have reports of people getting sick, but the fact that we are seeing examples in the environment means that there is risk," said Gast. "Part of coping with risk is finding out what's there."

—Andrea Carter

This research is supported by the Ocean Life Institute and Coastal Ocean Institute at WHOI and the Woods Hole Center for Oceans & Human Health, funded by the National Science Foundation and the National Institute of Environmental Health Sciences.



Under a microscope, green stain lets WHOI biologist Matt First easily track the journeys of bacteria within living organisms.