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A Plenitude of Ocean Life

A new census of the sea is revealing that microbial cells thrive in undreamed-of numbers. They form an essential part of the food web.

By Edward F. DeLong

Picoplankton
Photo by Edward F. DeLong

The *Polar Duke*, our ice-worthy Norwegian vessel, was immobilized—beset, to use the correct nautical term—by enormous sheets of sea ice. It was early August 1995, late winter in Antarctica, and the two-meter skin of frozen seawater that enveloped us was a seasonal expression of the Southern Ocean. Our destination was Palmer Station, a research station run by the National Science Foundation and situated on Anvers Island, off the Antarctic Peninsula. Evidently, though, our group of American scientists and support staff had set out just a little too soon. It took ten days for a change in wind and the breakup of the ice pack to free the ship, but by then we were low on fuel and forced to return to Chile to be resupplied. When we finally made it to Palmer Station, we were a month behind schedule. Only two months were left of our field season, and that was spent largely on cross-country skis, hauling sleds laden with carboys full of seawater.

So went the first visit of my research group to Antarctica. Our aim was to search out and quantify the range and biomass of a peculiar group of microorganisms known as archaea. The wisdom of the day was that the critters should not be present at all in the cold, oxygen-rich waters of the Southern Ocean. But a sample of Antarctic seawater collected in early 1990 at Palmer Station, carried to California, and given to us for analysis suggested otherwise. We hoped to show that archaea were major players even below the pack ice.

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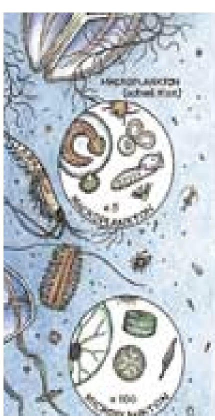
Archaea (originally dubbed archaebacteria) were not even recognized as a separate branch of life until the 1970s, when the microbiologist Carl R. Woese and his colleagues at the University of Illinois at Urbana-Champaign made a thorough analysis of their ribosomal RNA. This kind of RNA, which plays a role in protein synthesis, occurs in the small structures called ribosomes that exist in every known kind of cell. Because of its ubiquity, ribosomal RNA can serve as a kind of universal bar code for all organisms, placing them in proper historical relation to one another on a single evolutionary tree. Woese concluded that Archaea is one of three major evolutionary branches of life, as deeply rooted as Bacteria and Eukarya. (Eukarya, whose cells contain a nucleus and other structures, encompass plants, animals, fungi, and protists—protozoa, algae, and lower fungi.)

Apart from their evolutionary heritage, archaea appeared to have one thing in common: they thrived in extreme environments. At the time of our expedition, we knew some lived in saline lakes five times saltier than the ocean; some lived in anaerobic (oxygen-free) habitats, where even trace amounts of oxygen would prove lethal; and some lived in hot geothermal environments that would cook most organisms to a crisp. Among them was *Pyrolobus fumarii*, which could grow in anaerobic deep-sea hydrothermal vents at temperatures as high as 235 degrees Fahrenheit.

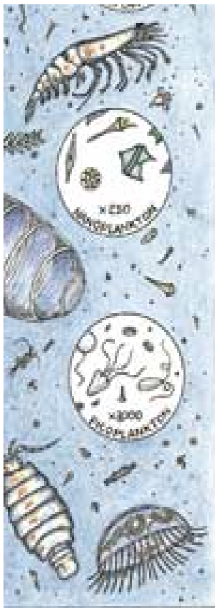
Our surveys of the frigid, aerobic Antarctic waters turned up archaea in great and unexpected numbers. Indeed, we have learned that cold-adapted cousins of heat-loving archaea appear to be flourishing in marine waters both shallow and deep and at all latitudes—polar, temperate, and tropical. They turn up in the guts of abyssal sea cucumbers and in sediments at the bottom of the sea. Quantitative surveys now show that archaea comprise between 20 and 30 percent of all the microbial cells in the ocean.

The discovery and enhanced understanding of so many new microbial groups stems not only from the quest to look in new places. Modern-day microbe hunters also have new, high-tech tools for identifying and counting microbial life. In the past the method of choice had simply been to culture a sample of, say, seawater and then see what grew. Although that approach is still being perfected, many cells stubbornly refuse to grow under laboratory conditions. The new techniques, some based on the tricks of molecular biology, enable biologists to find out what is in the samples by direct observation.



Plankton, sea life that drifts with the currents, ranges from the macroscopic to the microscopic. The so-called

Microbial life is proving to be far more diverse than cultured samples could suggest. A lot of the newly recognized life in the oceans is so small that its size is reflected in its name: picoplankton. The plankton comprises the floating “wanderers” of the sea, single-celled and multicelled plants and animals (including many immature larval forms) that move primarily by drifting with the currents [*see illustration at left*]. Anything smaller than 0.05 millimeter but larger than 2.0 microns,



picoplankton comprises between 0.2 and 2.0 microns across. Anything smaller (such as a virus) is part of the so-called femtoplankton.

Illustration by Patricia J. Wynne

capable of passing through fine-mesh nets, is considered nanoplankton (the prefixes “nano-” and “pico-” do not literally correspond to such measurement units as the nanometer or the picometer; they arise instead from naming traditions in marine biology). The picoplankton comprises the smallest cells, ranging between 0.2 and 2.0 microns across (between 1/500th and 1/50th the diameter of a human hair).

Until the 1970s, picoplankton was thought to be an insignificant element of the marine microbial food web; its biomass seemed much too low to play a primary role. But estimates of the numbers of microscopic planktonic organisms climbed dramatically in the late 1970s, when the so-called epifluorescence microscope was developed. This instrument, coupled with the use of fluorescent dyes that cause individual microbial cells to glow under ultraviolet light, enables the cells to be easily seen and counted. Technically, the process is an easy one. You simply add the dye, which binds to DNA in a sample of seawater, wait five minutes, collect the seagoing microorganisms on a filter, and observe them under the microscope. It is now known that the density of microorganisms ranges from tens of thousands per milliliter in the deep ocean to millions per milliliter in the energy-rich waters near the surface.

One might object that such a technique could not distinguish live cells from a lot of dead detritus floating around in the water. Studies in the early 1980s, however, which drew on biomedical techniques to measure the synthesis of DNA and protein, showed that marine picoplankton can double in biomass every day or so. So the cells observed with fluorescent dyes are very much alive and metabolically active. (In fact, the only reason the seagoing populations of picoplankton stay roughly constant is that protist predators are busily grazing on them at about the same rate as the picoplankton reproduces.)

The metabolic activity within the huge biomass of picoplankton represents a massive flow of carbon and energy. Some of the carbon is given off as carbon dioxide gas, but much of it remains locked up in organic molecules that help sustain the rest of the food web. Particularly important to the carbon cycle as well as to the entire oceanic food web are the microorganisms that live at or near the ocean’s surface: the forests of the sea are microscopic.

It has been known for some time that the top 600

Picoplankton comprises the

feet of the water column in the oceans is a region of intense photosynthetic activity. Carbon dioxide is combined with the energy of sunlight to produce a rich food harvest that supports all the other inhabitants of the ocean's surface, and most denizens of the deep as well. As recently as twenty-five years ago, all that productivity was credited to eukaryotic algal species, including diatoms, dinoflagellates, and their relatives. That now turns out to have been a faulty conclusion that arose from a major oversight.

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Shortly after epifluorescence microscopy was developed, the first of a new kind of photosynthetic microorganism was discovered: marine picoplanktonic cyanobacteria of the genus *Synechococcus*. Biologists were already familiar with cyanobacteria—they used to be called blue-green algae—because some kinds collect into so many individuals that they are visible in the aggregate. But the new cyanobacteria were much smaller, more abundant, and far more widely distributed than any previously known kind of "algae."

Like plants and genuine algae, cyanobacteria possess a kind of chlorophyll—so-called chlorophyll *a*—that enables them to "fix" carbon in the presence of sunlight, that is, to remove the carbon atoms from carbon dioxide gas and incorporate them into organic molecules. In the process the cyanobacteria give off oxygen, as do all plants that contain chlorophyll. Unlike plants, though, cyanobacteria lack a second kind of chlorophyll, known as chlorophyll *b*, which in concert with chlorophyll *a* helps plants capture light.

But cyanobacteria do harbor certain other pigmented proteins that help them harvest light energy. The proteins, known as phycobiliproteins, fluoresce red under the epifluorescence microscope, and that is how the new, tiny cyanobacteria were so easily detected and enumerated. By 1979, John B. Waterbury of the Woods Hole Oceanographic Institution in Massachusetts and John McNeil Sieburth of the University of Rhode Island on Narragansett Bay had shown that *Synechococcus* was extremely abundant in coastal and open-ocean environments, reaching densities greater than 100,000 cells per milliliter. Later experiments showed that at certain times and places these cells can be responsible for as much as half of the primary production of food in the ocean.

Chemical transformations, such as those in the nitrogen cycle and carbon cycle, depend on an entire community of microorganisms; no single species can carry out all

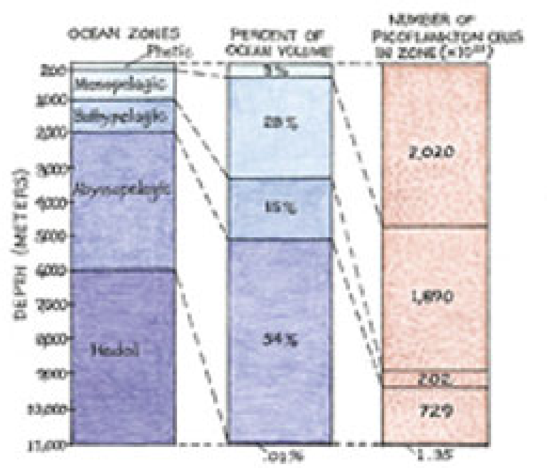
Then, in the late 1980s, the oceanographers Sallie W. Chisholm of the Massachusetts Institute of Technology and Robert Olson of Woods Hole discovered another small (less than a micron in diameter), red-fluorescing of them on its own.

kind of cell that was even more abundant than *Synechococcus*.

The new cells—in size, also a kind of picoplankton—were eventually cultured and isolated in the laboratory and given the genus name *Prochlorococcus*. They turn out to be closely related to *Synechococcus*, but the two genera differ in their pigment composition. Chisholm and her coworkers at MIT have now also determined the entire genome sequences of two *Prochlorococcus* strains, which represent high- and low-light-adapted "ecotypes." The low-light-adapted strain has significantly more genes than the high-light strain, perhaps because it needs more accessory proteins to efficiently gather light that is in short supply.

Field experiments have now shown that in the open ocean *Prochlorococcus* cells reach concentrations of hundreds of thousands per milliliter of seawater. In fact, *Prochlorococcus* constitutes half of the total chlorophyll-based biomass in the ocean. So picoplankton, once thought to be sparse and functioning mainly to recycle organic matter back into plant nutrients, proves to be much more central to the carbon cycle. The fact that picoplanktonic cells circulate in such vast numbers and are grazed upon by protists means that they supply nutrients directly to larger organisms. This microscopic portion of the food web has been dubbed the "microbial loop."

In our laboratory at the Monterey Bay Aquarium Research Institute, my colleagues and I are exploring a new technique of archiving the genomes of microorganisms en masse. The idea is to get a better understanding of the genetic, biochemical, and physiological properties of the organisms, as well as of their natural history.



Although the photic zone represents a small percentage of the ocean's volume, it contains the highest concentration of picoplanktonic cells. The smallest ocean zone by volume is the Hadal, named after Hades for its great depth.

Illustration by Patricia J. Wynne

Large DNA fragments, as long as 200,000 base pairs, are gathered higgledy-piggledy from mixed microbial populations and then cloned to create, in effect, an archive of microbial genetic diversity. Such a "library" serves as a repository of all the genes and genomes present in the original microbial population that was sampled. We can quickly search such libraries for the presence of particular genes—and by extension, the presence of the proteins and metabolic functions that the genes encode. We can also screen our libraries for markers that identify just which species of microorganism the genes belong to. In addition, proteins encoded by individual genes can be readily produced, making it possible to study their structure, function, and role in the natural world.

Unexpectedly, when we created one of our libraries, we discovered a previously unknown kind of photoprotein. The protein molecule, which came from the genome of a widespread planktonic bacterium, absorbs light of a characteristic wavelength—much as does rhodopsin, the light-sensitive pigment in the human eye. Indeed, the new photoprotein is chemically related to the rhodopsin family. And we have shown that, like rhodopsin, it can convert light into energy usable by the microbial cell. The function it serves for the bacteria—whether, for example, it enables them to fix carbon dioxide the way plants do, or is used to garner more energy for other cellular purposes—remains an open question.

Searching in Monterey Bay, from which the original genetic sample had come, we found that the novel form of rhodopsin occurs in natural communities of marine picoplankton. Further surveys in the oceans from Antarctica to Hawaii revealed that variants of the photoprotein exist virtually everywhere, in varying colors. In deep waters the photoprotein is "tuned" to absorb the blue wavelengths of light most abundant there. In shallower waters, it absorbs the more energetic green light available at the surface. It never fails: every time we dip into the living ocean, we find something new.

From the study of fossils known as stromatolites, a residue of the larger forms of cyanobacteria, biologists have long known that microorganisms have played key roles in the natural history of the Earth. Cyanobacteria were among the early actors on the stage of life; their capacity for photosynthesis and the oxygen they generated forever altered the global environment. They essentially paved the way for the evolution of other forms of life. Given the abundance of newly discovered cyanobacteria, one can only begin to appreciate what an important role they continue to play in the carbon cycle.

And the cyanobacteria exemplify just one way that marine microorganisms support the biosphere. Bacteria, archaea, and other microorganisms are also vital to the nitrogen cycle: they break down organic nitrogen to produce ammonia; they convert ammonia to nitrate, an essential plant nutrient; and they recycle nitrate into other nitrogen-containing compounds in oxygen-poor zones such as marine sediments. Some cyanobacteria can even synthesize organic

We hardly notice the jobs microbes do when everything is in balance and seemingly normal. It's only when something breaks down—when, say, excess nitrate in runoff waters creates a noxious algal bloom—that we begin to pay attention.

nitrogen compounds used to build new cells from simple nitrogen gas. In short, without microorganisms, nitrogen wouldn't cycle at all, and neither would most other elements.

It is important to recognize that such transformations depend on an entire community of microorganisms; no single species can carry out all of them on its own. Their interrelations are fantastically complex, forming systems that have been tuned by evolution in ways that work together. Therein lie the reasons for much of our ignorance about them. When they are going about their jobs, when everything is in balance and seemingly normal, we are least likely to notice them. It's only when something breaks down—when, say, excess nitrate in runoff waters creates a noxious algal bloom—that we begin to pay attention.

Microorganisms have been our planetary engineers, the biological stewards of the Earth, for as long as the world has had oceans, at least 3.5 billion years. They still have a lot to teach us.

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