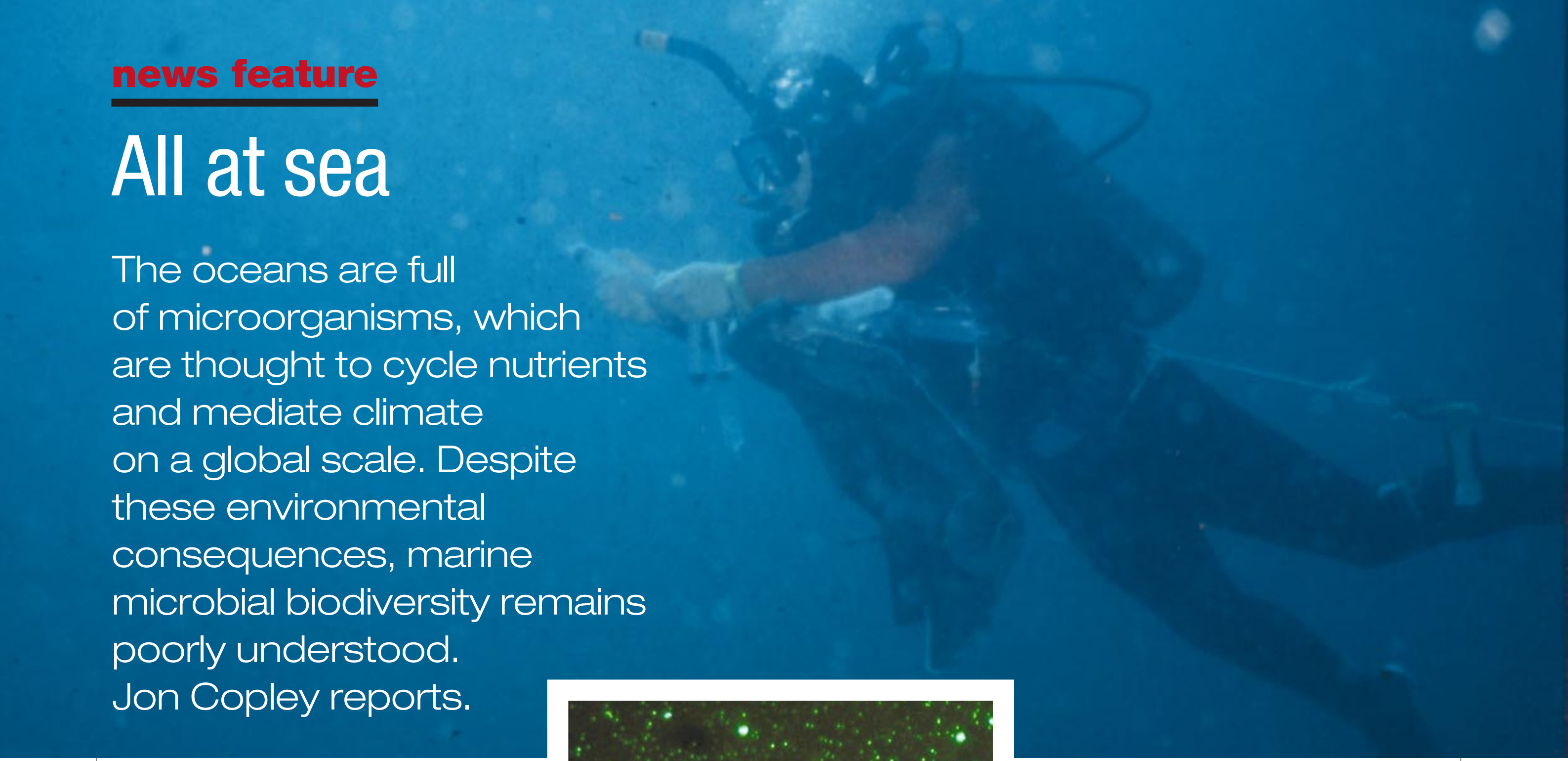


news feature

All at sea

The oceans are full of microorganisms, which are thought to cycle nutrients and mediate climate on a global scale. Despite these environmental consequences, marine microbial biodiversity remains poorly understood. Jon Copley reports.

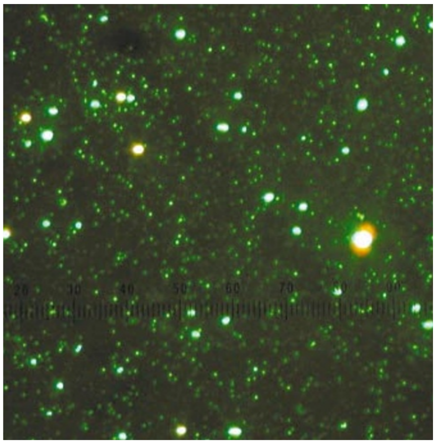
When it comes to mind-boggling numbers, marine microbiologists can give anyone a run for their money. The oceans are brimming with more than 3×10^{28} bacteria — that's about 100 million times more cells than there are stars in the visible Universe. But the real shock is that we have little idea what most of them are doing.

Only a tiny fraction of ocean-dwelling microorganisms have ever been cultured in the laboratory. Yet a better understanding of these microbes may be crucial to revealing how our planet works. "The role that the ocean plays in structuring the Earth's climate is largely driven by microorganisms," says Peter Burkill of the Plymouth Marine Laboratory in southwest England.

Around the world, researchers such as Burkill are now using a burgeoning range of techniques to sift through the myriad marine microorganisms. They hope to identify the key players and understand how these microbes influence the climate through their role in the cycles of elements such as carbon and nitrogen. Along the way, researchers are finding an unappreciated diversity of microbial life — and even new types of photosynthesis.

Microbial cells in the oceans are divided among three domains of life. There are the eukaryotes — single cells or simple colonies of cells whose nuclei are wrapped in membranes — such as green algae. Among the remainder — called prokaryotes — genetic and physiological differences define two further domains: the bacteria and the archaea. In addition, the oceans are teeming with viruses, most of which infect marine bacteria.

Archaea were first discovered in extreme environments such as the deep-sea vents that pour forth scalding sulphurous water from the ocean floor. They were thought to be restricted to such niches until researchers, including Jed Fuhrman of the University of Southern California in Los Angeles[1,2] and Ed DeLong of Monterey Bay Aquarium Research Institute in Moss Landing, California[3], found them to be widespread in the ocean. "They make up typically 40% of deep-sea organisms — and the deep sea is by far the largest habitat on Earth," says Fuhrman.



Send in the marines: researchers such as Ed DeLong (pictured) are finding fresh traits among oceanic microbes (inset). Looking like stars, these organisms outnumber their stellar counterparts.

C. GOTTSCHALK, UCSB; INSET: J. FUHRMAN

Active life

Rather than being inert refugees expelled from deep-sea vents, most of these cells are metabolically active members of the plankton. Fuhrman has shown that the cells can mop up amino acids from vanishingly small concentrations in their surroundings[4], and Markus Karner, then at the University of Hawaii in Honolulu, and his colleagues last year showed that the microbes contain plenty of ribosomal RNA — indicating that that they are busy making proteins[5].

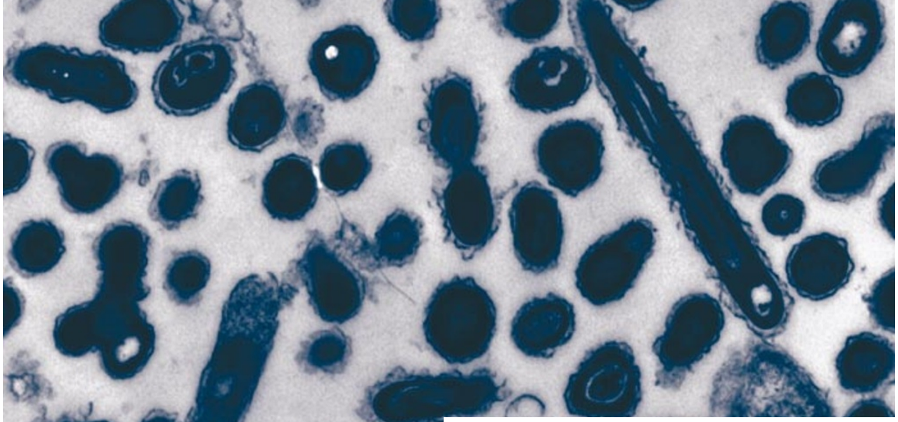
Although the prevalence of active archaea may have been a revelation, even the partially familiar can still yield surprises at sea. One might think that by now marine biologists understood photosynthesis — the use of light energy to make sugars — in the oceans. But abundant new forms of photosynthesis and phototrophy — the generation of chemical energy from light — have recently been found among marine bacteria. Paul Falkowski of Rutgers University in New Brunswick, New Jersey, and his colleagues came across one of them by accident.

Falkowski's group was hunting for photosynthetic microbes called α-proteobacteria in the waters around deep-sea vents, reasoning that the incredibly faint glow from the vents might just be sufficient to support photosynthesis. The team used an infrared fast-repetition-rate fluorometer, which flashes pulses of light lasting fractions of a microsecond. These flashes cause chlorophyll and other photosynthetic pigments present to fluoresce. By measuring the wavelength and duration of this fluorescence, it is possible to deduce how much and what type of pigment is actively involved in photosynthesis.

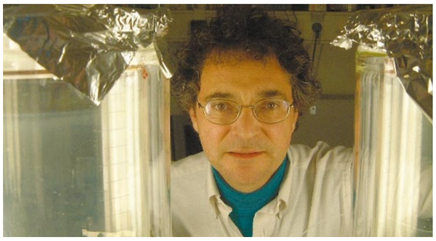
No signs of photosynthesis were detected in water samples from deep-sea vents, but the team also tested their equipment on surface waters. Looking at the behaviour of the microbes in surface-water samples, the researchers estimated that α-proteobacteria account for up to 5% of the photosynthesis taking place[6]. "When we first saw these signals in the upper ocean, we thought there was something wrong with our machines," recalls Falkowski. But unlike traditional practitioners of photosynthesis, such as eukaryotic algae or cyanobacteria, the α-proteobacteria do not produce oxygen.

Jed Fuhrman spotted widespread archaea.

I. HEWSON



P. FALKOWSKI



G. GORSKY

Paul Falkowski has found unusual photosynthetic behaviour in some marine proteobacteria.

Instead they use oxygen to synthesize their chlorophyll and grow. This unusual variant of photosynthesis, called 'aerobic, anoxygenic', was previously thought only to occur among the α-proteobacteria in places such as beach sands and on the surfaces of seaweeds.

Falkowski and his colleagues have found the α-proteobacteria wherever they have looked in the surface waters of the Pacific and the Atlantic. "We have 30 strains of them in culture," says Falkowski. "We believe they comprise approximately 10% of all the bacteria in the ocean." The photosynthetic α-proteobacteria are relatively abundant in vast circular currents known as gyres, which tend to contain low levels of the dissolved organic matter that non-photosynthetic bacteria rely on as a source of energy.

Fixed return

Photosynthetic organisms usually 'fix' carbon by using light energy to turn inorganic carbon, often in the form of carbon dioxide, into sugars. Falkowski's α-proteobacteria also fix carbon, but in an unusual way. "They have a funny kind of metabolism called photoheterotrophy," he says. In other words, the microbes use light energy to help them break down organic matter, but they fix some inorganic carbon as well.

Carbon fixing is key to the way in which oceans absorb CO_2 — and so affect climate. But simply adding more carbon-fixing organisms to the ocean does not necessarily increase its capacity to absorb atmospheric CO_2. This also depends on the processes that remove fixed carbon from surface waters. But the new bacteria add a fresh layer of complexity to the flow carbon takes through the ocean.

Much of the carbon fixed in the ocean ends up as dissolved organic matter, released when microorganisms die. This organic matter can be used by other microbes that may in turn be consumed by yet other microbes. In this way, the organic matter eventually finds its way to the tiny creatures known as zooplankton — a process known as the microbial loop. The zooplankton subsequently die and some of their biomass sinks to the ocean floor, locking away the fixed carbon.

Some carbon is lost at each step in the microbial loop, as the organisms involved respire and release CO_2. So knowing the length of the microbial loop is important for estimating the efficiency with which the ocean can process carbon.

The photosynthetic α-proteobacteria complicate matters further because they both use up dissolved organic matter in respiration, and can also add to it by fixing CO_2. "They're actually making a loop within a loop," says Falkowski. This recycling of carbon keeps it in surface waters, rather than sinking to the ocean depths. That is good news for organisms that can use it, but — depending on the relative rates of the processes involved — potentially bad news for anyone hoping that the oceans will quickly put excess atmospheric CO_2 out of harm's way.

In this issue of *Nature*[7], DeLong's group, working with researchers at The Institute for Genomic Research in Rockville, Maryland, show that the α-proteobacteria are far from the only marine microbes to practise aerobic, anoxygenic photosynthesis. After analysing bacterial gene sequences from seawater samples, they concluded that a multitude of distantly related bacteria can do the trick, including some unrelated to any known species. Other details of the physiology of these microbes remain unknown — clearly there is much work to be done before their effect on carbon cycling and other environmental processes is fully understood[8].

In earlier work, DeLong and his colleagues came across another surprise when trawling through the genome of a member of a group called the γ-proteobacteria. They spotted a gene very similar to one that codes for the pigment rhodopsin in archaea[9]. Rhodopsin absorbs light, a reaction used by some archaea to drive their proton pumps — part of the molecular machinery used by cells to make chemical energy. But this type of phototrophy had never before been seen in bacteria.

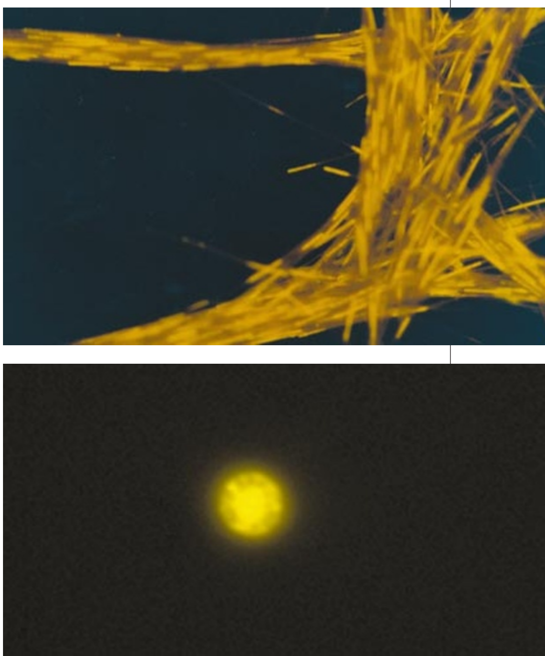
DeLong's group has since shown that this new phototrophic pigment, dubbed proteorhodopsin, is present in bacteria from the coastal waters of Monterey Bay, the central North Pacific and the Southern Ocean[10]. The researchers have also identified different versions of the pigment tuned to the different lighting conditions in the waters in which the bacteria involved are found. As with Falkowski's α-proteobacteria, this previously overlooked form of solar power may be widespread in the oceans — and significant for the flow of carbon through them.

In the loop

The jury is still out on whether the proteorhodopsin-containing bacteria actually fix carbon. But regardless of the verdict, the bacteria's ability to generate chemical energy using sunlight reduces their need to obtain energy by oxidizing dissolved organic matter. So these bacteria may also make their mark on the microbial loop.

Nitrogen is another vital element in the ocean, stimulating the microbial growth. "How much CO_2 can be removed by the oceans through photosynthesis is dependent on fertilization by nutrients such as nitrogen," says Jonathan Zehr of the University of California, Santa Cruz. Unfortunately, the most abundant form of nitrogen — dissolved N_2 gas — is useless to most marine life. It first has to be trapped in a biologically accessible form by nitrogen-fixing organisms.

The cyanobacterium *Trichodesmium*, which sometimes forms filamentous colonies just visible to the naked eye, has long been known to use a nitrogenase enzyme to



Y.-B. CHEN

G. STEWARD

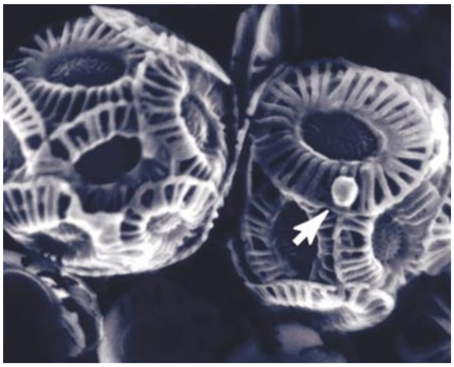
Gas traps: filamentous *Trichodesmium* (top) and single-celled cyanobacteria both fix nitrogen.

convert N_2 into ammonia. But it is not abundant enough to account for the amount of nitrogen being fixed in the ocean, suggesting that there are other nitrogen-fixers out there.

Zehr and his colleagues have found messenger RNA coding for part of the nitrogenase enzyme in several strains of smaller unicellular cyanobacteria 3–10 micrometres in diameter, and have shown that these microbes can fix radiolabelled N_2 (refs 11,12). Once again, these newly appreciated microbes seem to be widespread in surface waters[13]. Their role in nitrogen fixation could be equal to that of *Trichodesmium*, Zehr suggests.

Zehr is convinced that yet more nitrogen-fixers are waiting to be described. "We're finding other culprits," he says. Falkowski has a hunch that his photosynthetic α-proteobacteria could be involved. And Zehr has just received funds from the US National Science Foundation to examine symbiotic relationships in which the new nitrogen-fixing cyanobacteria live inside larger single-celled algae. "These may turn out to be the next really exciting story," he says.

SEAWIFS, NASA/ORBIMAGE



W.WILSON

Wipe out: tiny marine viruses (arrowed, inset) kill the alga *Emiliania huxleyi*. They help to control its milky blooms (main picture), and in doing so influence cloud formation.

Variety show

Beyond the bacteria and archaea, molecular techniques are now revealing a hitherto unrecognized diversity of small eukaryotes in the picoplankton — cells less than 3 micrometres across. By analysing ribosomal RNA gene sequences, researchers led by David Moreira of the Miguel Hernández University in Alicante, Spain, have unearthed a host of new eukaryotes in the deep sea of the Southern Ocean[14,15]. "I have a feeling that we will realize that their diversity is comparable to that of the prokaryotes," predicts Moreira, who recently moved to the Pierre and Marie Curie University in Paris.

Using the same approach, researchers led by Daniel Vaulot of the Roscoff Biological Station in Brittany, France, have found a similarly diverse assemblage in the surface waters of the Pacific[16]. The eukaryotes are important grazers of other picoplankton, says Vaulot.

The picoplankton also contains photosynthetic eukaryotes, whose taxonomy and physiology is poorly known — although a European programme called PICODIV, coordinated by Vaulot, may soon start revealing their secrets.

At the very bottom of the size spectrum are the marine viruses. "In an average seawater sample you'll get around a million bacteria per millilitre, but you've usually got about 10 million viruses," says Willie Wilson of the UK Marine Biological Association in Plymouth. Most infect bacteria, preventing any one type from dominating the plankton — if the population of a particular bacterium explodes, it becomes a target for a viral epidemic that culls its numbers.

Viruses can also transfer genes between different organisms. "That kind of genetic transfer has the potential to have a profound effect, even if it's a one-in-a-billion event," says Fuhrman. "It's a subject that is wide open for discovery." One intriguing possibility is that the proteorhodopsin gene found in DeLong's γ-proteobacteria may have been transferred from archaea in this way.

Bloom and bust

The viruses also attack single-celled algae. In unpublished work, Wilson has isolated one virus that pervades the huge blooms of *Emiliania huxleyi* that can turn patches of the North Atlantic milky white. "We have 10 different strains of the virus from coastal waters in the English Channel," he says, adding that the viruses are one factor causing the eventual death of the algae in these blooms.

When infected cells die, they release dissolved organic matter. So viruses might influence the microbial loop and the carbon cycle. "There's no question that they play a part in the big picture," says Fuhrman.

Wilson has also shown that when viruses kill off algal blooms, large quantities of dimethlysulphide (DMS) gas is released into the atmosphere. DMS stimulates cloud formation, increasing the proportion of solar radiation reflected back into space — which can have a dramatic cooling effect on climate.

In further unpublished studies, Burkill and his colleagues have found that some α-proteobacteria are also involved in this process, converting dimethylsulphoniopropionate (DMSP) released by dying algae into the cloud-forming DMS. "We've identified the main route by which DMS is produced in sea water," Burkill claims. His team used flow cytometry, originally developed to sort and study human cancer cells, to pinpoint the DMSP-converting microorganisms.

Findings such as this illustrate the gains being realized now that a diverse set of biological tools is being applied to the study of marine microbial biodiversity and the roles that the microbes perform in ocean ecosystems. "What's fantastic is that the community has been able to bring to bear such a range of techniques," says Burkill. Even so, marine biologists are still barely scratching the surface of understanding the microbial processes that determine the cycling of carbon and nitrogen in the oceans — information that will be crucial to determining the influence that marine microorganisms exert over our climate. ■

Jon Copley is a freelance writer in Southampton, UK.

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