Deep-sea hospitality: an enigmatic worm and its symbionts

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Forty years ago, scientists discovered an animal at the bottom of the ocean that changed forever how we view life on this planet. Abundant, thriving animals were not expected in the deep sea, due to the very low levels of organic carbon that sink down from above. The giant tubeworm *Riftia pachyptila* pays little attention to this problem, having renounced its mouth and digestive system during the course of evolution. Instead, this animal relies on a partnership with internal bacteria that act as a built-in source of nourishment and, in exchange, *Riftia* performs unparalleled physiological feats.

Extraordinary physiology

Deep-sea hydrothermal vents are home to a variety of invertebrate species, many of which host chemosynthetic bacteria in unusual symbiotic arrangements. *Riftia pachyptila* is probably the most successful invertebrate host living at the vents along the East Pacific Rise. These gigantic, gutless worms must meet the metabolic needs of their symbionts. The bacteria are housed inside their cells, snuggled between mitochondria and a nucleus, within a novel organ called the trophosome (literally meaning 'feeding tissue') not observed in any other animal lineage. Unlike other animals, *Riftia* must take up and transport carbon dioxide (CO_2) and hydrogen sulfide (HS-) to their symbionts. Typical animals respire CO_2, having produced this waste product from the oxidation of organic carbon – like humans breathing out CO_2 after eating scrambled eggs for breakfast. Further, HS- is an incredibly toxic molecule that can (and often does) cause death when present in very low concentrations in much larger animals, such as humans. On top of this, the primary metabolism of the internal symbionts- sulfide-driven chemosynthesis – i.e. conversion of CO_2 into organic carbon using energy from the oxidation of reduced sulfide compounds – results in the massive production of protons (H^+). This would normally drive the internal pH of these worms to fatally acidic conditions, were it not for their souped-up ability to efficiently get rid of these ions. All things considered, being a host to intracellular chemoautotrophic symbionts is an intimate relationship that, while critical to survival, requires physiological compromise and a great deal

A large clump of red-headed *Riftia pachyptila* tubeworms, two miles deep in the Gulf of California, soaking up the sulfide emanating from nearby hydrothermal vents. © Monterey Bay Aquarium Research Institute of effort by this unusual, yet incredibly successful, deep-sea worm.

Life in the abyss

By the 1930s, early accounts of life in the deep sea (based mainly on eyewitness accounts by Dr William Beebe, a pioneer submersible diver) dispelled the notion that the deep sea was an extreme, inhospitable, wasteland, virtually devoid of life. Nothing, however, prepared scientists for the revelation made on-board the *R/V Lulu* in February 1977, off the Galapagos Islands. In 2500 metres of water, they saw thriving, dense, animal communities that rivalled tropical rainforests in biomass – despite the absence of light, deep at the bottom of the ocean. The discovery of life near these underwater volcanoes ('hydrothermal vents', as they are called), fuelled almost entirely by chemicals gushing from superheated fluids, forever changed our idea of the outer limits of life on this planet.

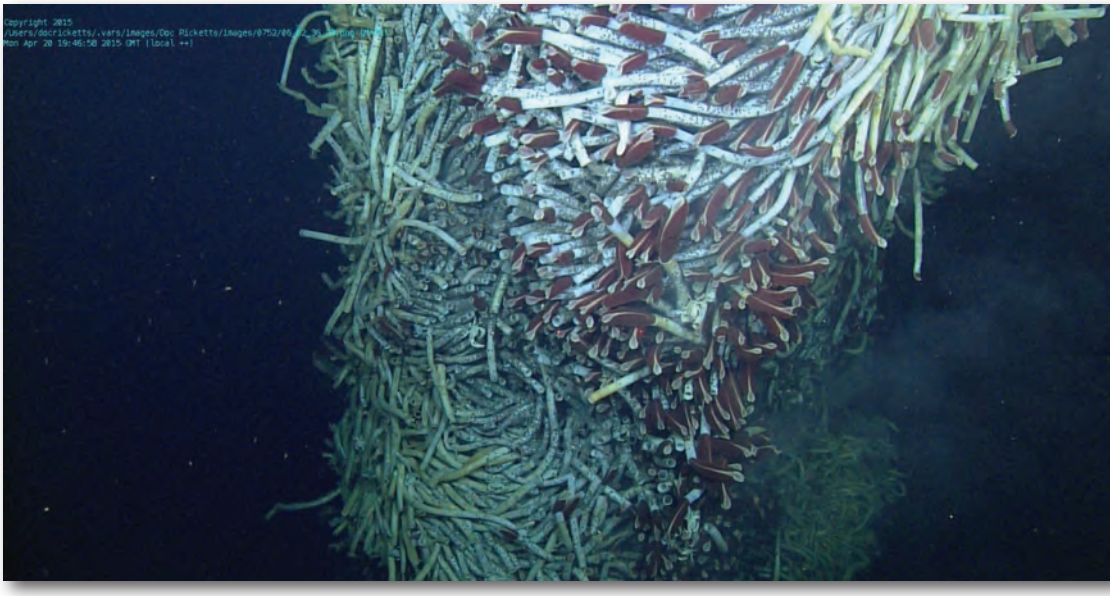
Enabled by a secret partner

When dense populations of worms and bivalves were first reported from deep-sea camera tows along the Galapagos Spreading Center, they were described as filter-feeding organisms, subsisting by gathering up the very little organic carbon that remains suspended in seawater at a depth of 1.5 miles. The fact that these worms had no mouth or digestive system must have cast doubt on these initial assumptions. But, it was not until groundbreaking examinations by two research teams ultimately uncovered the secret to the success of these unusual animals – a symbiotic merger with chemoautotrophic bacteria. One research group (at Scripps Institution of Oceanography) took an enzymatic approach and tested *Riftia* tissues for enzymes found only in metabolic pathways of autotrophic organisms (ex. bacteria and plants), including ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) and adenosine phosphosulfate reductase. High activities of these and other enzymes suggested the presence of a second organism, an autotrophic bacterium, within the tissues of the enigmatic deep-sea worms. Simultaneously, a research team at Harvard University used electron microscopy to observe bacteria, packed like sardines within the unusual trophosome tissue of *Riftia*. These unprecedented findings put to rest the idea that somehow *Riftia* could sustain very large populations based solely on the uptake of small particulate carbon, and kicked off a scientific revolution of sorts, unveiling the powerful and pervasive nature of bacterial partnerships with marine invertebrates.

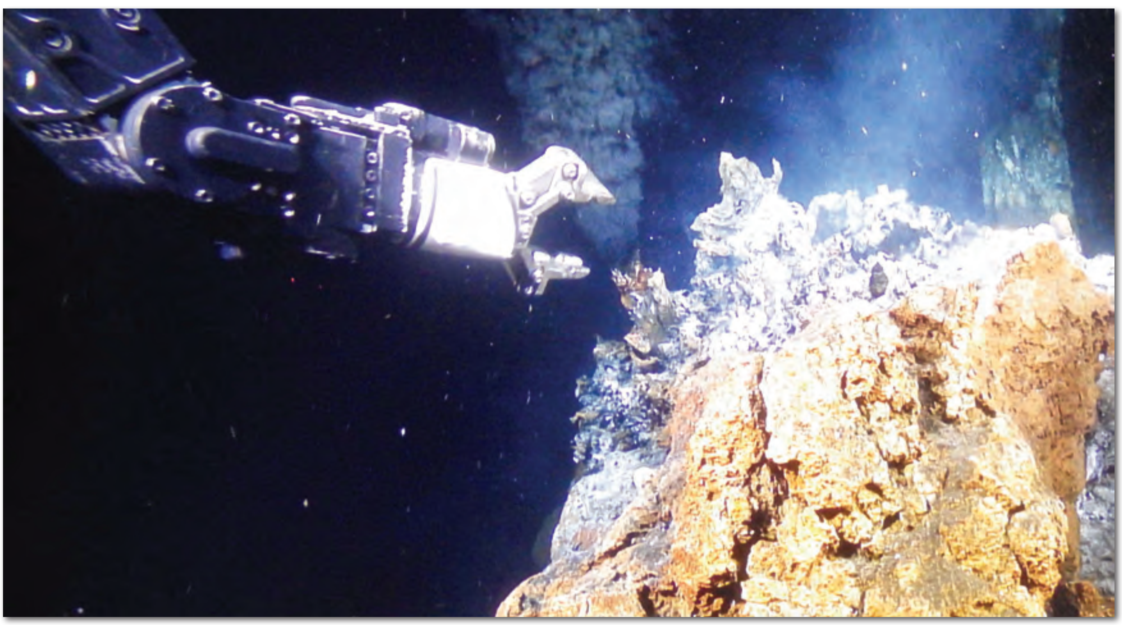
A worm that acts like a plant

Autotrophy is a term given to an organism that creates its own organic carbon from inorganic carbon (the literal translation of autotrophy is 'to generate one's own food'). Numerous studies in the 1990s confirmed that the bacterial symbionts of *Riftia* require so much CO_2 to support not only their own organic carbon production, but also that of their fast-growing worm host. The animal as a whole exhibits net CO_2 uptake from the surrounding seawater, thus behaving like a plant. *Riftia* worms accomplish this CO_2 uptake by virtue of precision

An artist's rendition of *Riftia pachyptila*, showing internal bacterial symbionts, exposed by the action of a predatory crab. These worms take up carbon dioxide and hydrogen sulfide across their respiratory plumes. The gases are transported to the symbionts, in residence in a special tissue called the trophosome, where they produce organic carbon for the worm, and waste products such as protons and sulfate ions. Artist: Naomi Field pH control (they maintain a blood pH differential 1 pH unit above the slightly acidic environment, equivalent to 10-fold more hydrogen ions) and an enzyme known as carbonic anhydrase, that catalyses the interconversion of CO_2 and bicarbonate ions in seawater, thereby facilitating movement of inorganic carbon into the animals. Based on recent genomic and proteomic analyses, we now know that the *Riftia* symbionts are also able to use two different pathways for the conversion of CO_2 into organic carbon: the well-known Calvin–Benson cycle and a reverse version of the citric acid cycle, known as the reductive tricarboxylic acid cycle. Why the bacterial symbionts of *Riftia* have such carbon fixation versatility is not known, but scientists suspect this may be a way to respond to rapid environmental changes, including sulfide availability, and to accommodate enzymatic sensitivities to compounds such as oxygen.



A towering assemblage of *Riftia pachyptila* tubeworms, some of which no longer appear to be alive (at left), likely due to changes in the flow of life-giving hydrothermal fluids. © Monterey Bay Aquarium Research Institute



The manipulator arm of the *R.O.V Doc Ricketts* prepares to sample a piece of the billowing hydrothermal chimney, nearby small clumps of *Riftia pachyptila* tubeworms.© Monterey Bay Aquarium Research Institute

Human-like haemoglobins allow sulfide transport

In addition to CO_2, the bacterial symbionts must also be supplied with ample amounts of HS- in order to produce enough ATP to fuel the expensive fixation of carbon. These worms actually take up the charged form of HS- via surface transport proteins. As mentioned, sulfide is not only an excellent energy source, it is incredibly toxic, swiftly poisoning many metabolic pathways, including aerobic respiration. *Riftia* accomplishes this proverbial dance with death by using haemoglobin much like our own, to bind and transport sulfide. Haemoglobin is a type of respiratory pigment (i.e. a coloured protein involved in gas exchange) found in many animal lineages, and some plants. It is thought that this molecule originally evolved to bind and detoxify gases, including oxygen, but was co-opted as a taxicab for oxygen delivery to the tissues of active, larger animals. Haemoglobin is also very attracted to HS-, in addition to oxygen, which is partly the reason for sulfide toxicity in animals. *Riftia* possesses not one, but three different haemoglobin molecules that travel within circulatory vessels, moving sulfide from the surface of the feathery anterior plume to the bacteria-containing trophosome. Once at the location of the symbionts, deep inside the animal, a molecular hand-off occurs; the haemoglobin releases both the oxygen and sulfide it carries. The symbionts then use these two compounds together to generate ATP via substrate level phosphorylation and via traditional oxidative phosphorylation through an electron transport chain. In this way, toxic sulfide is kept bound for its entire residence time inside of the animal and is never free to poison either partner.

Internal ion balance is critical

The maintenance of a proper internal pH is critical for all living organisms. However, in the case of *Riftia*, it is paramount, given that this makes possible the uptake, unusual for an animal, of both CO_2 and sulfide. In experiments on-board the research ship, worms are collected from the seafloor by submersible and immediately put into steel vessels capable of pressurization to *Riftia's* optimal pressure – 3000 psi (i.e. pounds per square inch; imagine the equivalent of 10 baby elephants sitting on every square inch of your body). Exposure of these experimental worms to chemical inhibitors of specific ion transport processes revealed H^+-ATPases as the dedicated mechanism by which *Riftia* counteract the build-up of acidic end-products of symbiont metabolism (they maintain an alkaline internal pH of 7.0–7.4, versus the surrounding environment, of pH 6.0). Total ATPase activities measured in *Riftia*, in response to the internal production of protons by their beneficial symbionts, are three times higher than those observed in other marine worms, thus making them champions of the acid-base balance Olympics.

More mysteries await

Riftia provided the first ever demonstration of a symbiosis between a marine invertebrate and chemoautotrophic bacteria. However, shortly after this discovery, numerous other chemosynthetic symbioses were revealed in both the deep-sea and shallow water sulfide-rich habitats, including seagrass beds, mangroves, estuaries and sewage outfalls. These novel symbioses were considered exceptional within the animal kingdom until the advent of molecular tools that allowed the rapid assessment of bacteria associated with all kinds of habitats and organisms, including other animals, plants and even some protozoans (single-cell eukaryotes). Even today, novel bacterial symbioses are being discovered at a rapid pace – symbionts that can use hydrogen as an energy source, in addition to HS-; marine bivalves that have sulfide-utilizing autotrophic symbionts, as opposed to their typical wood-degrading heterotrophic symbionts; and even a recent discovery of symbioses in both mussels and sponges based on short-chain alkanes as energy sources. What began as a niche field of research now provides fundamental knowledge about the crucial cooperation among organisms, including bacteria and humans. Without a doubt, the discovery of giant tubeworms at the bottom of the ocean is one of the greatest discoveries of the 20th century. ■

Dr Shana Goffredi is an Associate Professor of Biology at Occidental College. Her research interests mainly concern beneficial symbiotic partnerships between bacteria and marine invertebrates. For 20 years, she has been exploring the deep ocean. She focuses on the physiology and biochemistry of deep-sea symbiotic systems, within the context of ecological questions and how environmental influences dramatically affect their functioning. At Occidental College, she teaches courses on zoology, microbial diversity and symbiosis. Dr Goffredi's funding has come from the National Science Foundation, and she has published in journals such as Proceedings of the Royal Society, Environmental Microbiology *and* Science. *Her BS degree is in Biology/Marine Science from the University of San Diego and her PhD is in Ecology, Evolution and Marine Biology from UC Santa Barbara. Email: sgoffredi@oxy.edu.*

Further reading

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