

# **THE DISCOVERY OF HYDROTHERMAL VENTS**

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## **Physiology and Biochemistry of the Hydrothermal Vent Animals**

by

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Reprinted from *Oceanus*, Vol. 27, No. 3, 1984

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# Physiology and Biochemistry of the Hydrothermal Vent Animals

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For a physiologist or a biochemist interested in how organisms manage to thrive in different environments, a useful starting point is to ask, "What types of stresses present in the environment may necessitate adaptive responses by the organism?" By examining how organisms have responded, through evolution, to the conditions in their habitat, a biologist can gain understanding of two critical features of organisms: their abilities to tolerate habitat conditions, and their capacities for total biological activity (rate of metabolism, reproduction, et cetera).

If we approach hydrothermal vent animals from this perspective, we find no shortage of environmental stresses to consider in our analysis. From our perspective as terrestrial creatures living under one atmosphere (atm) of pressure, with ample oxygen to breathe, contact with noxious gasses avoidable (for the most part), and with plenty of sunlight in our environment to drive carbon-dioxide fixation via photosynthesis, the vents seem a highly stressful habitat. At the depths where the Galápagos and 21 degrees North (East Pacific Rise) vent sites occur—2,500 to 2,600 meters—ambient pressures range between 250 and 260 atm (hydrostatic pressure rises by about 1 atm for each 10 meters of depth). These pressures are high enough to severely perturb many physiological systems. The waters flowing from the vents, which the vent animals must "breathe," are rich in hydrogen sulfide ( $\text{H}_2\text{S}$ ), a molecule highly toxic to almost all living systems. Hydrogen sulfide, even at concentrations less than one-thousandth of those found in some vent animals, poisons aerobic respiration, the metabolic pathway most animals rely on for extracting energy from their food.

Getting food in the first place can be a problem for organisms living deep in the oceans. Far from the surface, the vents are well removed from the zone in which photosynthetic fixation of carbon occurs (the euphotic zone). Virtually all photosynthesis in the oceans is carried out by small green algae in the upper 100 to 200 meters of the water column. Thus, any foodstuffs supplied to the vent animals from photosynthetic production must

travel almost 2,500 meters (approximately 8,000 feet) down the water column. And, based on studies in which sediment traps were employed to discern how much reduced carbon can reach the deep ocean from the euphotic zone, it seems unlikely that more than a small percentage of the reduced carbon compounds produced photosynthetically in the euphotic zone can reach depths near 2,500 meters.

Knowing the locations of the vent sites and the composition of the waters emitted from the vents, one might predict that hydrothermal vents would be relatively impoverished in animal life, as, indeed, the typical deep sea appears to be. This expectation is not borne out; rather, hydrothermal vent communities contain some of the densest aggregations of animal life found on land or in the sea. Vastly more abundant than animals from the typical deep sea, many vent organisms also strike us as being odd. They have anatomical, physiological, and biochemical properties that set them off dramatically from most other animals.

Probably the oddest among the vent animals is the large tube worm, *Riftia pachyptila* Jones, the largest known member of phylum Pogonophora (see page 47). *Riftia* may reach approximately one meter in length and several centimeters in diameter. Yet its lack of a mouth and digestive system immediately raises the question, "How does this animal obtain its nutrition?" *Riftia*, like the largest, most populous bivalve molluscs at the vents, lives only where a steady flow of sulfide-rich water occurs; that is, the animals that constitute the largest fraction of the vent animal biomass have chosen for their habitat the most stressful niche available at the vents!

## Hydrogen Sulfide

The feature of the vent environment that probably has most influenced the evolutionary path taken by the vent organisms is a molecule I have already mentioned, hydrogen sulfide. From the standpoint of its roles in metabolism, hydrogen sulfide is at once good and evil. Certain features of this molecule help account for the abundance (and

some of the most unusual characteristics) of vent animals. Hydrogen sulfide is a highly reduced molecule, meaning that, if the proper metabolic machinery is present, a great deal of energy can be obtained when hydrogen sulfide is oxidized. This potential can only be realized, of course, if the organism feeding on sulfide avoids being poisoned by it.

The ability to exploit the energy contained in the molecular bonds of hydrogen sulfide is restricted to certain types of bacteria, which possess the enzyme systems needed to oxidize sulfide and to use the energy released to fuel their metabolisms. The energy released drives the Calvin-Benson cycle, the biochemical pathway used by photosynthetic organisms and certain nonphotosynthetic bacteria to fix carbon dioxide. Nonphotosynthetic bacteria with this potential—species able to use sulfide plus species able to tap the energy of other inorganic sources—have what is termed a chemolithoautotrophic metabolism. Literally, these bacteria “eat” reduced inorganic chemicals like hydrogen sulfide, which, in effect, takes the place of the sunlight used by photosynthetic organisms. Thus, given a supply of energy-rich inorganic chemicals, chemolithoautotrophic bacteria can do in the dark what light-requiring photosynthetic organisms like marine algae can do only in the presence of sunlight: achieve net fixation of carbon dioxide and synthesize reduced carbon compounds (sugars and the like). This provides nourishment for the organisms performing these syntheses and can nourish organisms that feed on these primary producers.

The role of sulfide-oxidizing bacteria in the vent ecosystem is crucial (see page 79). Bacteria form the base of the vent foodchain, and provide much, if not all, of the reduced-carbon compounds needed by the vent animals. How, then, do the animals feed on the vent bacteria? The answer is quite complicated; biologists are only now beginning to understand the nutritional relationships between chemolithoautotrophic, sulfide-oxidizing bacteria and the vent animals that depend for their sustenance on these bacteria.

One type of feeding may be a simple process of “grazing.” Motile invertebrates, such as small crustaceans, may crawl over the rich bacterial mats and “graze” on these “fields” of bacteria, much as a herbivore feeds on green plants. Another form of exploitation of the bacteria is more subtle: chemicals released from the bacteria when they die mix with the seawater and serve as a source of dissolved organic molecules for the soft-bodied vent invertebrates. Soft-bodied marine invertebrates have outstanding abilities to extract organic molecules (such as amino acids) from seawater. These dissolved organic molecules are important in the animals’ nutrition. Unfortunately, we do not know what types and quantities of dissolved organic molecules are present in the vent waters, so their contribution to the needs of the vent animals is unclear. Future expeditions to the vents will measure the types and quantities of organic molecules in the vent waters, and also

determine how effectively these molecules are taken up by the vent animals.

### Symbiosis

Perhaps the most important way in which bacteria contribute to the vent animals’ dietary needs is through symbiotic interactions. In a symbiotic system, two different organisms live in close association for the mutual benefit of both. For example, many corals house small photosynthetic algae within their tissues, thereby gaining an “in house” supply of photosynthetic products. The symbiotic algae benefit too: they receive anchorage in a stable, sunlit habitat, and have direct access to the carbon dioxide released via respiration from the animal tissues.

In *Riftia pachyptila*, and in the vent bivalves, including the large white clam, *Calyptogena magnifica*, and the vent mussels, sulfur bacteria may play a role analogous to that of the photosynthetic algae in corals. Colleen Cavanaugh of Harvard University and her colleagues first showed that the trophosome tissue of *Riftia pachyptila* is densely populated with bacteria (Figures 1 and 2). The trophosome is a soft tissue filling much of the internal body cavity of pogonophoran tube worms. It is thought to play a major nutritional role for the worms. Biochemical studies by Horst Felbeck of the Scripps Institution of Oceanography demonstrated that the trophosome bacteria could oxidize sulfide and use the energy released to drive the Calvin-Benson cycle. Subsequent work by Cavanaugh has shown that the vent bivalves house large numbers of bacteria in their enormous gills, and Felbeck and co-workers have shown that the symbionts of the bivalves, too, have the enzyme systems needed to exploit the energy of sulfide for driving net carbon dioxide fixation (Figure 3).

Although we basically understand these animal-bacteria symbioses, vast amounts of additional study will be required before we will be able to answer the following questions. First, how heavily do the animals depend on the nutrients produced “in house” by their symbionts? Are the symbionts providing the dominant fraction of food, vitamins, et cetera, for the animals, or does the uptake of dissolved and/or particulate organic matter from external sources also make an important contribution to the animals’ nutrition? For *Riftia pachyptila*, which lacks a mouth and digestive tract (Figure 1), it is tempting to speculate that most, if not all, of the animal’s dietary needs could be supplied by the symbiotic bacteria. However, judgment on this point must be suspended until more work is done.

Second, what types of organic molecules are transferred from symbiont to host and from host to symbiont? How does the supply of nutrients from bacteria vary with the host animal’s nutritional needs? What types of nutrients might the animal “feed” its bacterial colony? For example, might the animal “feed” its symbionts molecules that can signal, to the bacteria, what the host’s nutritional requirements are? Lastly, to return to the important question of how the animals cope with the toxic

effects of sulfide, we can ask how the animal supplies its sulfide-oxidizing bacteria with enough hydrogen sulfide to fuel the bacterial chemolithoautotrophic metabolic system and yet not poison the metabolism of host and bacterial tissues.

### Survival Strategies

Given a clean sheet of paper, how might one design an organism that can live in the presence of potentially toxic concentrations of hydrogen sulfide? Such a bioengineering approach might involve several strategies. One idea is to build around the organism a protective coat impermeable to sulfide. Unfortunately, if the animal must take in sulfide to fuel its bacterial symbionts' metabolisms, such a simple defensive strategy cannot be the answer. A second possibility is to redesign the respiratory proteins that are the primary locus of sulfide poisoning. For instance, the respiratory enzyme cytochrome c oxidase, which is involved in the use of molecular oxygen in aerobic respiration, but is poisoned by very low concentrations of sulfide, might be modified (over evolutionary time) to gain an insensitivity to sulfide.

A sulfide-tolerant animal might run its metabolism with anaerobic pathways of energy generation. In this way, the sulfide poisoning of aerobic respiration would not be a problem. However, the energy yield from degrading foodstuffs through anaerobic metabolic pathways is vastly lower (as much as 90 percent lower) than the energy yield obtained when these same foodstuffs are fully combusted to carbon dioxide and water via aerobic pathways. Thus, the strategy of giving up aerobic respiration seems suboptimal.

A fourth strategy requires a high degree of evolutionary novelty, for it entails the "invention" of a new type of protein, a "sulfide-binding protein," that could bind sulfide extremely tightly and prevent it from entering the mitochondria, intracellular organelles where the sulfide-sensitive respiratory enzymes are located. Such a sulfide-binding protein could keep the levels of free (unbound) sulfide extremely low, well below the toxic threshold. At some point, of course, the sulfide-binding proteins would have to unload their bound sulfide, so the challenge facing this type of protein is not only to tie up sulfide, but also to release it at an appropriate site, under appropriate conditions.

A fifth possible approach is the inclusion of sulfide-detoxifying enzymes on the superficial layers of the animals. These enzymes could form a "peripheral defense" by detoxifying any sulfide not rapidly taken up by the symbiotic bacteria or bound by the sulfide-binding proteins.

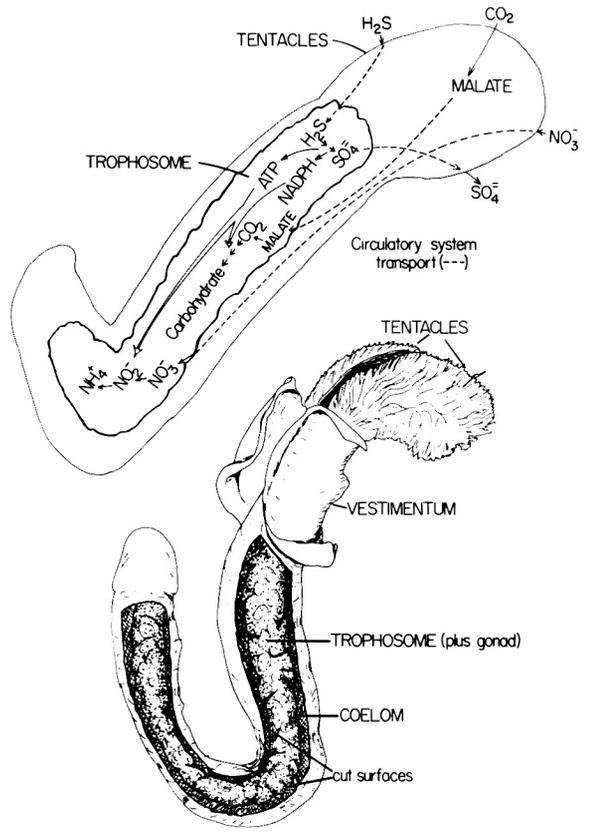


Figure 1. Metabolic and anatomical models of the pogonophoran tube worm, *Riftia pachyptila*. The lower drawing illustrates the anatomical organization of the worm. The trophosome tissue contains large numbers of chemolithoautotrophic bacteria, supplied with hydrogen sulfide ( $H_2S$ ) by the well-developed blood system of the worm. Only the tentacles are in direct contact with the ambient seawater. The upper figure shows the essential biochemical transformations thought to occur in this symbiosis. Sulfide ( $H_2S$ ) is oxidized to sulfate ( $SO_4^{2-}$ ), which is returned to the environment. Some of the energy released during the oxidation of sulfide is used to produce the biological "energy currency" compounds, ATP and NADPH, which in turn are utilized to drive the net fixation of carbon dioxide ( $CO_2$ ) and the reduction of nitrate ( $NO_3^-$ ) to ammonia ( $NH_4^+$ ).  $CO_2$  is thought to be trapped in the four-carbon compound, malate, for transport to the bacteria in the trophosome.

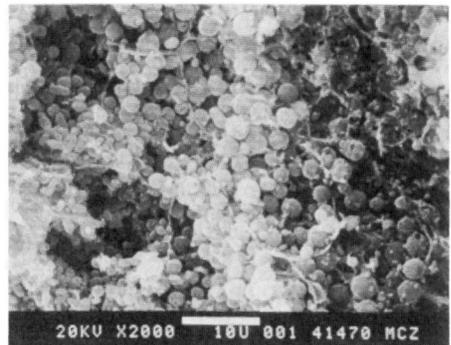


Figure 2. Scanning electron micrograph, right, showing symbiotic bacteria from the trophosome of *Riftia pachyptila*. (Scanning electron micrograph courtesy of Colleen Cavanaugh of Harvard University)

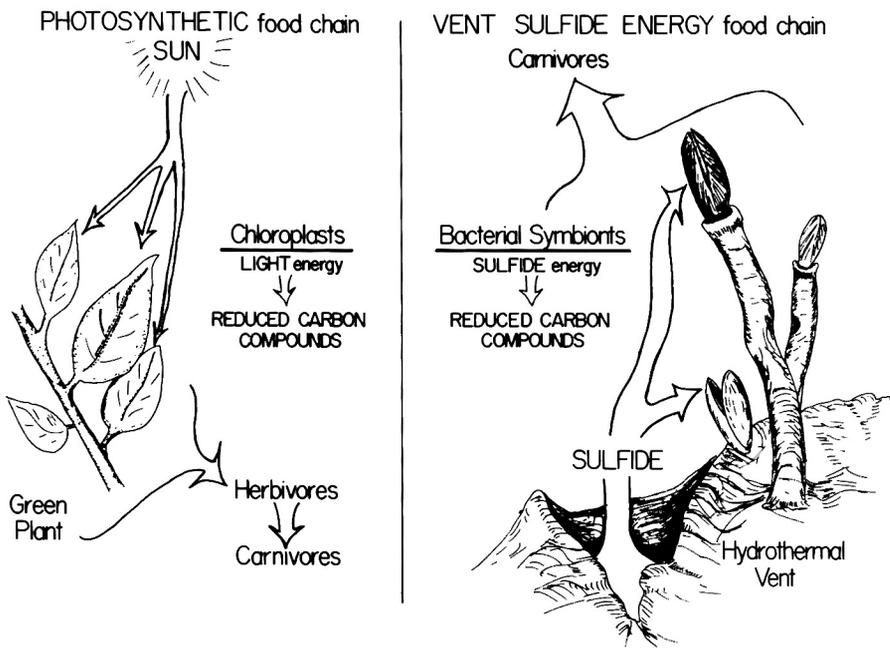


Figure 3. A comparison of food chains, sunlight-driven versus sulfide-driven. In photosynthetic food chains, sunlight is used in the chloroplasts (chlorophyll containing organelles) to drive  $\text{CO}_2$  fixation via the Calvin-Benson cycle. In the vent tube worm and clam, energy released from sulfide oxidation by bacterial symbionts is used to power the Calvin-Benson cycle for synthesis of reduced carbon compounds.

### Exploring *Riftia's* Adaptations

Our best data come from studies of *Riftia pachyptila*, which uses at least two of these strategies to good advantage. To determine whether *Riftia pachyptila* relies, as most animals do, on efficient aerobic metabolic pathways for extracting energy from its food, Steven Hand (University of Southwestern Louisiana), Mark Powell (a graduate student at Scripps), and I measured the ability of the tentacle of *Riftia* to consume oxygen. We found, indeed, that the tissue respired rapidly. Thus, a shift away from efficient aerobic metabolism to anaerobic processes was not operative. However, in contrast to other animals studied, we found that the oxygen consumption of tentacle tissue from *Riftia* was not strongly inhibited by sulfide. Is this ability to respire in the presence of sulfide a reflection of sulfide-insensitive respiratory enzymes in *Riftia*? Not at all: the cytochrome c oxidase of *Riftia* was found to be just as sensitive to inhibition by sulfide as the cytochrome c oxidases of animals from non-vent habitats. We observed, however, that the sensitivity of the cytochrome c oxidase of *Riftia* to sulfide was strongly dependent on the extent to which we had purified the enzyme; in other words, on the extent to which other types of proteins had been removed from our preparations. The most highly-purified preparations of the enzyme were the most sensitive to sulfide, a result suggesting that something in the tentacle tissue used as our source of enzyme was able to protect the enzyme from sulfide. Because the tentacle is highly vascularized, as evident from its bright red color, we reasoned that a blood-borne factor could be protecting the cytochrome c oxidase system. To test this conjecture, we added a minute amount of blood to a preparation of sulfide-inhibited enzyme.

To our satisfaction, we found that full activity of the cytochrome c oxidase system could be restored by the addition of even very small amounts of blood from the worm.

We conducted these experiments aboard the *R/V New Horizon*, as part of the Oasis 82 Expedition to the 21-degrees-North vent site, in April and May of 1982 (other vessels taking part in the expedition were *R/V Melville* and *R/V Alvin/Lulu*). At the same time, James Childress and Alissa Arp (University of California, Santa Barbara) were working aboard the *R/V Melville*, examining sulfide transport by the blood of *Riftia*. They had just discovered a protein in the blood of the worm able to bind sulfide very tightly, possibly serving as a sulfide-transport protein, much as hemoglobin transports oxygen. When we compared notes, it became obvious that we had focused in on the same protein, starting from two different directions. This one type of protein, never before observed, could be doing at least two critical things for the animal and its bacterial symbionts. The sulfide-binding protein, as Childress and Arp named their discovery, appeared to be able to extract sulfide from the seawater and transport the molecule via the animal's bloodstream to the bacteria in the trophosome. The sulfide-binding proteins are protective, too. Because there are high concentrations of the sulfide-binding proteins in the blood of *Riftia*, and because these proteins can bind sulfide extremely tightly, levels of free sulfide in the blood are kept too low to pose a threat to the animal. As well, sulfide-binding proteins may exist within the cells of the animal or bacteria, possibly serving to withdraw sulfide from the blood of *Riftia* to "feed" to the sulfide-oxidizing machinery of the bacteria. Ongoing studies of

these proteins may further illuminate their contributions to the symbiosis.

As vital to *Riftia*'s survival as the sulfide-binding proteins appear to be, the worm needs another defense against sulfide. This is the "peripheral defense" strategy mentioned earlier. Studies by Mark Powell and myself have shown that the very outer layer of the sausage-like bodywall of *Riftia* (Figure 4) contains enzyme systems capable of rapidly oxidizing any free sulfide that enters the cells. This "peripheral defense" may be extremely important in poorly vascularized tissues, like the body-wall musculature, where a rich blood supply (with plenty of sulfide-binding protein to tie up sulfide) is not present (Figure 5).

### Other Vent Fauna

Thus far, I have discussed primarily the adaptations of *Riftia pachyptila*, an emphasis reflecting the large share of attention that the biologists working in the vent program have given this unusual worm. In fairness to the other members of the vent fauna, however, it must be emphasized that many other animals found in the sulfide-rich outflows have solved the same adaptational problems. Studies of the respiratory metabolism of the vent bivalves and crustaceans by Kenneth L. Smith, Jr. (Scripps), and James J. Childress and Thomas Mickel (University of California, Santa Barbara) have shown that these species, too, sustain aerobic respiration, generally at levels similar to those of related species from shallow-water habitats. This is interesting in light of the findings made by Childress, Smith, and others that pelagic animals from typical deep-sea regions have extraordinarily low metabolic rates. High pressure alone is not responsible for the low metabolic rates of pelagic deep-sea animals. The metabolic rates of the vent animals no doubt reflect the abundance of food present at the vents, relative to the nutrition available to other deep-living pelagic species.

How the other vent animals deal with sulfide toxicity is not clear. For many species, simple avoidance of sulfide-rich waters may preclude the need for defenses against the toxic effects of sulfide. Perhaps the distribution patterns of animals at the vents reflect, in part, the different species' abilities to cope with sulfide. Only certain species, for example *Riftia* and the large bivalves, are clustered in the flow of sulfide-rich waters; other animals, such as the "spaghetti worm," appear to occur only outside the zones of high sulfide concentrations. On our next trip to the vents, we will investigate whether the more peripherally occurring species lack sulfide detoxifying systems.

Mark Powell and I have found that the "peripheral defense" noted in the body-wall musculature of *Riftia* appears to occur in the huge foot of *Calyptogena magnifica* (Figure 6). This clam typically lives along cracks in the seafloor through which sulfide-rich waters issue, its foot engorged with blood and extended deeply into the crack. Thus, the clam's foot must be protected from sulfide inhibition of metabolism.

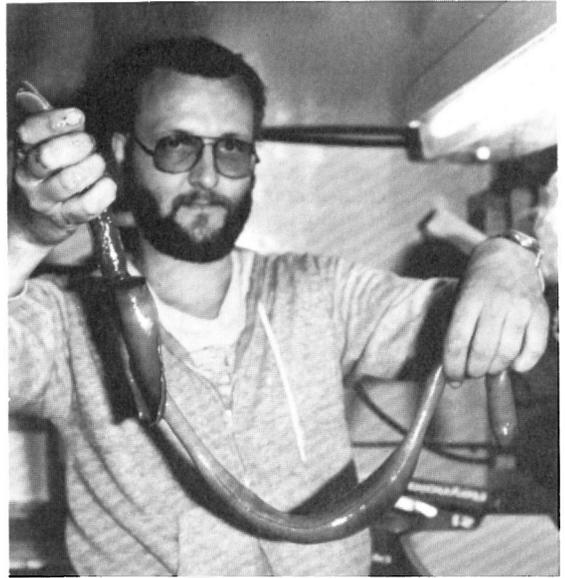


Figure 4. Horst Felbeck of Scripps holding a large specimen of *Riftia* that has been removed from its white tube. (Photo by Steven C. Hand)

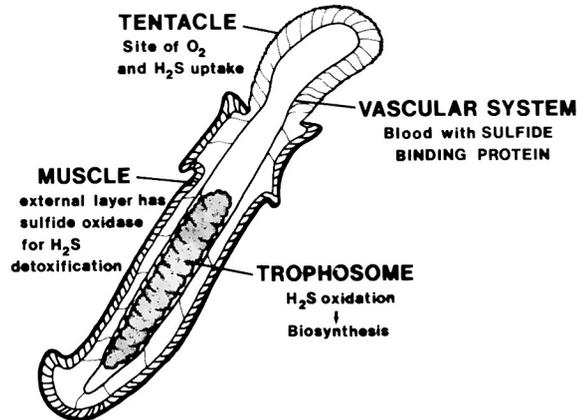


Figure 5. How hydrogen sulfide is handled by *Riftia pachyptila*. Sulfide ( $H_2S$ ) and oxygen ( $O_2$ ) are taken up through the tentacle and carried by the blood to the bacterial symbionts in the trophosome. During transport the sulfide is tightly bound to sulfide-binding protein, thus preventing toxic levels of free sulfide from occurring in the blood. The body-wall musculature has a superficial layer where sulfide-oxidizing activity takes place, possibly to detoxify any sulfide that penetrates into the musculature.

### Summary

From the perspective of the environmental physiologist and biochemist, the vent animals offer some vivid lessons concerning the adaptability of living systems. These animals have one of the most stressful habitats imaginable: high pressures; no light, therefore no photosynthetic productivity; and waters laden with toxic substances. Through evolutionary changes, the vent animals have met

these challenges, and can tolerate and even thrive in their unusual environment. These creatures are most aesthetic and fascinating for those biologists fortunate enough to be able to work with them.

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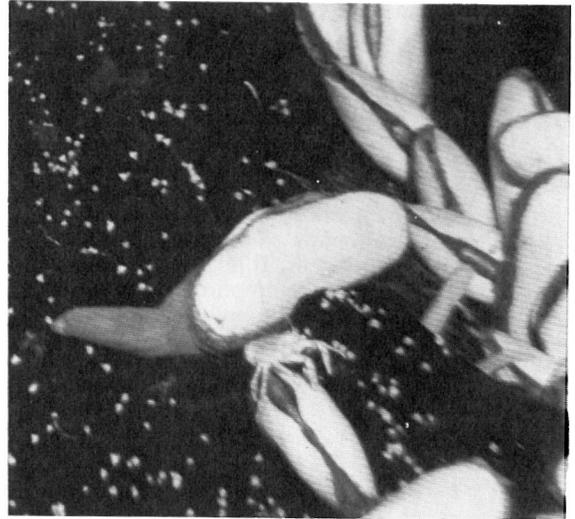


Figure 6. The large vent clam, *Calyptogena magnifica*, with its foot extended. The clam is found along cracks in the seafloor where sulfide-containing waters are emitted. The foot of the clam usually is extended down into the crack. (Photo by James J. Childress)