

Metazoans in Extreme Environments: Adaptations of Hydrothermal Vent and Hydrocarbon Seep Fauna

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ABSTRACT

Some of the most extreme environments where animals survive are associated with active vents and seeps in the deep sea. In addition to the extreme pressure, low temperatures, and lack of light that characterize the deep sea in general, a variety of other factors that are hostile to most animals prevail in these environments. Hydrothermal vent regions show extremes in temperature, areas of very low oxygen, and the presence of toxic hydrogen sulfide and heavy metals. Hydrocarbon seeps, though much cooler than vents, also have regions of very low oxygen and high hydrogen sulfide, as well as other potentially harmful substances such as crude oil and supersaturated brine. Specially adapted animals not only tolerate these conditions, they often thrive under them. In most cases this tolerance is due to a combination of physiological and behavioral adaptations that allow animals to avoid the extremes of their habitats and yet benefit from the chemoautotrophic production characteristic of these environments.

INTRODUCTION

Metazoans are multicellular animals and, as such, are more complex in their body plans and often have stricter physiological requirements than unicellular animals or bacteria. Individual cells in these organisms perform specialized tasks, and cells of similar function are typically organized into layers or compartments. In most cases, this means that materials cannot simply diffuse from the environment to all cells. Therefore, systems must be in place to transport materials throughout the body, to allow communication between different compartments, and to regulate the internal environment in which these processes occur. Strictly speaking, all known metazoans are heterotrophic and must depend on the oxidation of autotrophically produced organic carbon compounds for energy. This metabolic process can occur in the absence of oxygen (anaerobically). However, in the presence of oxygen (aerobically), the amount of energy harnessed per unit of fixed carbon oxidized increases dramatically. As metazoans often have relatively large body sizes and high energy demands, they require not only ample supplies of fixed carbon and oxygen but also environmental conditions under which their complex biochemical pathways may function. An extreme environment for a metazoan, then, may be one in which fixed carbon or oxygen is scarce or absent, or where environmental conditions—such as temperature, pressure, or the presence of toxins—hinder or prevent physiological processes.

The deep sea is a relatively inhospitable environment for metazoans: ambient temperature is constantly low (~2° C), pressure is high, light is absent, and organic carbon is scarce. Low temperatures may slow or impede many biochemical reactions and decrease the fluidity of lipids, a factor of primary importance to cell membrane function

(reviewed in Somero, 1998). High pressure can alter enzyme kinetics and efficiency, change protein structure (reviewed in Gibbs, 1997). The sheer distance of much of the ocean floor from a source of photosynthetic production makes it largely a nutrient-poor environment, where any carbon consumed by an organism must be strictly conserved (Sibuet, 1992; Gage and Tyler, 1996). A diverse array of metazoans have responded to this combination of environmental extremes by developing specialized proteins and lipids, capable of functioning under temperatures and pressures that would hinder shallower-dwelling species, and depressed metabolic rates and activity that conserve a limited energy supply (reviewed in Gibbs, 1997 and Somero, 1998).

Hydrothermal vent environments have the same high pressure and continuous darkness characteristic of the deep sea in general, but they differ in nutrient supply, temperature variation, oxygen concentration, pH, and levels of such potentially toxic chemicals as sulfide and heavy metals (Childress and Fisher, 1992). In active venting regions, entrained seawater is superheated by deep, subsurface, hot basalt, converting geothermal energy into the chemical energy of hot, highly reduced hydrothermal fluid (Jannasch, 1989). This superheated fluid rises through a system of interconnected subterranean cracks and fissures in the newly formed sea floor, resulting in multiple flow sites and flow manifestations at the surface. In areas where the effluent reaches temperatures up to 400°C and actively mixes with the cold oxygen-bearing ambient seawater, chimneys or mounds may form when chemicals rapidly precipitate out of solution. Hydrothermal fluids may also mix with cooler water as it rises through the basalt, emerging as diffuse flow fields at temperatures ranging from slightly above ambient to about 100°C (Delaney et al., 1992) (Figure 1).

Hydrocarbon seeps in the Gulf of Mexico provide a chemical environment similar to that of hydrothermal vents, though differing significantly in temperature, sulfide concentrations, and types of toxins. Sulfide in seep areas is produced when organic carbon (crude oil and natural gas) migrates to the sea floor from deep reservoirs. Sediment layers at the sea floor diffuse and retain the seeping oil, and sulfate reducers in the upper meters of sediment produce hydrogen sulfide. The upward migration of fluids at cold seeps is to some extent thermally driven (Kennicutt et al., 1992). Unlike the process in vents, however, this process is more passive, and does not result in vigorous mixing of seep fluid and ambient bottom water. Seep fluids are normally at ambient water temperatures by the time they reach the sediment/water interface. Sulfide levels found in emitted seep fluid are generally much lower and more stable temporally and spatially, than levels found in vents, (MacDonald et al., 1989; Julian et al., 1999). However, interstitial sulfide concentrations can be comparable to those found at hydrothermal vents (Kennicutt et al., 1989). Unlike

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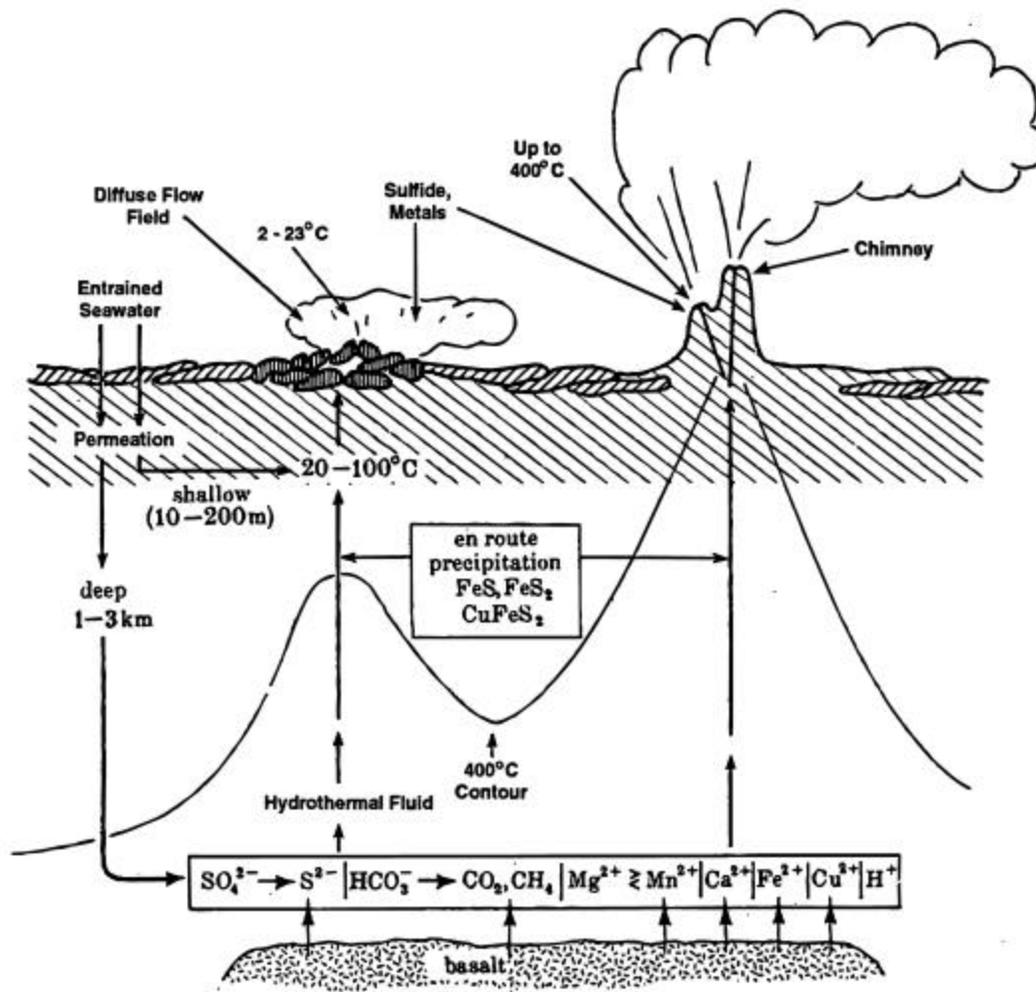


Figure 1. Hydrothermal Vents Manifest on the Sea Floor as Chimney Structures and Diffuse Flow Fields. *Superheated seawater carries highly reduced compounds to the surface, where they rapidly precipitate out of solution or fuel extensive biological communities based on chemoautotrophic production.* (Modified from Jannash, 1989)

the fauna in vent regions, fauna in seeps experience temperatures that do not vary appreciably from ambient deep-sea temperatures (~8°C). Methane and crude oil may also bubble through overlying sediments at these sites, and dense anoxic brines can form pools on the seafloor (MacDonald, 1990; MacDonald, 1998).

The abundant reduced chemicals found in both hydrothermal vent and hydrocarbon seep fluids, hydrogen sulfide in particular, can be used by chemosynthetic prokaryotes as an energy source for carbon fixation. For metazoan life, this offers the potential for a large food source more or less independent from the overlying photic zone (Jannasch, 1989). Most vent and many seep organisms rely entirely on this chemosynthetic primary production by actively feeding on free-living bacteria or by forming symbiotic relationships with chemosynthetic bacteria for the bulk of their food supply (Childress and Fisher, 1992; Kennicutt and Burke, 1995; Fisher, 1996). The sulfide-containing hydrothermal vent fluid on which primary production relies is hot, extremely deficient in oxygen, and laden with toxic chemicals. Moreover, sulfide and oxygen react spontaneously and do not coexist in significant

concentrations for significant periods of time (Millero, 1986; Johnson et al., 1986). The needs of the symbiont or free-living bacteria for sulfide and oxygen, and of the animal for additional oxygen, limit vent fauna to areas where hot, sulfide-bearing vent water and cold, oxygen-bearing ambient water actively mix (Childress and Fisher, 1992). Animals in such regions therefore experience rapid shifts in temperature that coincide with changes in oxygen and sulfide concentration. Seep fauna are also limited by access to sulfide and, as sulfide is present in areas of active seepage, are exposed to potentially toxic levels of hydrocarbons.

The extraordinarily high level of chemosynthetic-based primary production that is supported by venting and seepage is one of the outstanding differences between these regions and the average deep-sea environment. Metazoans are found at high densities in vent and seep regions, but they are of low diversity and have a high degree of endemism (Tunnicliffe et al., 1998; Sibuet and Olu, 1998). Organisms of taxonomic and functional similarity are found at both seeps and vents. It appears that vents and seeps are both areas of significant resources and of extreme environmental

demands that are largely exploited by a limited group of animals with specialized physiological and biochemical adaptations (Childress and Fisher, 1992; Fisher, 1996).

TEMPERATURE

Temperature variation is one of the striking characteristics of hydrothermal vent environments. Water temperature can range from 2°C to 400°C within a centimeter, and animals may have occasional brief contact with 100°C+ water (Chevaldonne et al., 1992; Delaney et al., 1992; Cary et al., 1998). In areas of diffuse flow, a vent animal may experience water temperatures of 2, 20 and 40+°C in rapid succession, or even simultaneously over the length of its body (Johnson et al., 1988; Cary et al., 1998). Additionally, at least one life stage of these organisms must be able to withstand extended periods of cold (~2°C) during dispersal.

Many biological structures, such as enzymes and lipid bilayer membranes, depend on a particular degree of molecular instability or fluidity, which is directly affected by temperature. Increasing temperatures can increase reaction rates and affect reaction equilibria through higher kinetic energy, and high temperatures can cause protein denaturation, resulting in complete and often irretrievable loss of function (Hochachka and Somero, 1984). Adaptation to the deep sea requires more “fluid” proteins and lipids to compensate for the stabilizing forces of high pressure and low temperature (Hochachka and Somero, 1984). High temperature, on the other hand, tends to destabilize molecules, and selection in such environments is for more stable forms. At vent regions, however, temperature can vary by almost 100°C within an animal’s habitat (Cary et al., 1998; Desbruyères et al., 1998). How, then, do vent organisms maintain proper function in such extremes?

As with all metazoan challenges, this threat can be met through morphological, physiological, and behavioral adaptations. Biochemically, changes that favorably affect reaction rate equilibria and molecular stability become fixed in a population. A number of molecules show a particularly strong correlation between functional and denaturation temperatures and the temperature of an organism’s environment (Hochachka and Somero, 1984). Molecules of particular interest are enzymes, collagen, and lipids. Enzymes quickly lose function above and below an optimal temperature. The subunits of the structural polymer collagen, the most abundant animal protein, often show a melting temperature (T_m) close to the upper lethal limit for an animal, though the polymer itself is somewhat more stable. Lipids of cell membrane bilayers must be both fluid and structurally coherent to form a functional membrane, a characteristic that is also very sensitive to temperature change (Hochachka and Somero, 1984).

Many hydrothermal vent animals must have a wide temperature tolerance (especially compared to ambient deep-sea fauna). Of particular interest are such chimney-dwelling polychaetes as *Alvinella pompejana*, *A. caudata*, and *Paralvinella sulfincola*, which live on newly formed vent walls very near the super-hot vent fluid. Photographic data from one such site show an alvinellid crawling over a temperature probe that is reading 105°C

(Chevaldonne et al., 1992). Temperatures of 20–80°C have been measured on surfaces colonized by *P. sulfincola* and *P. palmiformis* (Juniper et al., 1992), and temperature measurements taken in alvinellid tubes have recorded temperatures of 68°C, with spikes as high as 80°C (Desbruyères et al., 1998). These recordings have led some authors to conclude that alvinellids regularly experience such high temperatures, and that alvinellid tubes may open to vent fluid at the back, allowing warm vent water to flow outward over the animals (Cary et al., 1998).

Measurements of temperature effects on alvinellid proteins, however, indicate that these animals cannot survive body temperatures over 50°C for extended periods. For example, both *Alvinella pompejana* and *A. caudata* have hemoglobin that is unstable at 50°C, with highest oxygen binding affinities occurring at roughly 15° and 25°C, respectively (Toulmond et al., 1990). More striking are the melting temperatures of alvinellid collagens, which in *A. pompejana* are 40°C for cuticle collagen and 46°C for interstitial collagen (Gaill et al., 1991). Based on these measurements, these animals likely live at temperatures averaging 30 to 35°C rather than at the higher temperatures proposed by others (Childress and Fisher, 1992; Fisher, 1998). The conflict between observed temperature probe readings (Chevaldonne et al., 1992; Cary et al., 1998) and the *in vitro* physiological limits probably reflects both the steep temperature gradient that exists within alvinellid tubes and the difference between a temperature probe reading and actual body temperature. Even if these animals do not experience body temperatures of 60+°C, they live at temperatures much higher than ambient and may even take advantage of the large temperature gradient present over the lengths of their bodies—from 22°C near the gills to 60+°C at the body trunk (Cary et al., 1998) (see discussion of alvinellid hemoglobin).

Animal survival in extreme temperatures depends as much on behavior as on physical adaptations. Different species in vent fields have different environmental requirements, and they will either settle in or migrate to regions that meet their particular needs. Within specific habitats, animals may also have behavioral mechanisms for modifying environmental conditions to their benefit. *Paralvinella sulfoncola* colonizes young sulfide chimneys in the early stages of sulfide mineralization, where both temperature and sulfide levels have begun to decrease (Juniper and Martineau, 1995). Additionally, *P. sulfoncola* has been found to have a 2-mm-thick layer of FeS₂ below its tubes, which forms a barrier between hot vent water and cold sea water—a barrier that may be formed by the activity of the animal itself (Juniper and Martineau, 1995). Alvinellids, in general, may also actively cool their tubes by pumping in ambient seawater (Chevaldonne et al., 1991).

Though the vent animals described here likely withstand body temperatures that rival those of the most thermotolerant metazoans, the best-documented thermotolerant animals are found in other, more easily studied environments. Desert-adapted bees fly with a sustained internal temperature of 46°C (Willmer and Stone, 1997), and desert ants can survive even hotter temperatures.

The Australian ant *Melophorus bagoti* actively forages at soil temperatures above 70°C and has a critical thermal maximum of 56.7°C, surviving for one hour at 54°C (Christian and Morton, 1992). Unlike desert insects, however, vent organisms live in a world of rapidly shifting temperature extremes: within minutes, water temperatures vary in tens of degrees at a given location and in hundreds of degrees over centimeters. Vent organisms must therefore be adapted to extreme temperature variation as well as to extreme temperatures.

HYPOXIA/ANOXIA

Metazoan metabolic energy is produced via the release and transfer of electrons from reduced-carbon electron donor molecules to more oxidized electron acceptors. In aerobic metabolism, the electron acceptor is oxygen; in metazoan anaerobic metabolism, the electron acceptor is an organic molecule such as lactate or fumarate. Aerobic respiration yields 36 ATP/mol glucose oxidized, while anaerobic respiration yields 2-8 ATP/mol glucose, depending on the pathway and electron acceptor utilized (Fenchel and Finlay, 1995).

Metazoans rely primarily on the efficient, high-energy output of aerobic respiration to maintain a normal level of metabolism. Metazoans forced to use inefficient fermentation for energy production over extended periods need a large and constantly replenished food source to maintain normal bodily function and reproduction, a situation not common in nature, although endoparasites are a possible exception (Bryant, 1991). Oxygen is also involved in a number of critical biosynthetic pathways, and no metazoan has yet been conclusively documented to complete its entire lifecycle without its presence, though obligate anaerobe eukaryotic protozoa do exist (Fenchel and Finlay, 1995). Some nematode and oligochaete meiofauna, which occur deep in the sulphidic zone of sediments, may prove to be capable of growth and reproduction in the absence of oxygen (these environments have not yet been established as completely anaerobic) (Fenchel and Finlay, 1995). Yet numerous metazoans from diverse groups and environments (e.g., nematodes, annelids, amphipods, and goldfish) can withstand extended periods of anoxia (Bryant, 1991; Panis et al., 1996; Hagerman et al., 1997).

Vent and seep fluids are highly reduced and contain significant levels of sulfide. Oxygen in vent habitats varies inversely with temperature, and organisms in areas of actively mixing hydrothermal and ambient water may experience rapid fluctuations in both (Childress and Fisher, 1992) (Figure 2). At hydrocarbon seeps, oxygen concentration decreases with proximity to the substrate and with increased depth in the sediment (Kennicutt et al., 1989). Seep organisms colonize both brine pools and methane hydrates, which are habitats of particularly low oxygen (MacDonald, 1990; Fisher et al., 2000). Metazoans with chemoautotrophic sulfide-oxidizing symbionts at vent and seep sites require oxygen for aerobic respiration, as well as both oxygen and sulfide to power chemoautotrophic carbon fixation. This dual requirement can prove a challenge for metazoans that are dependent upon chemoautotrophic symbionts, because they will require substantial amounts of both oxygen and sulfide, two chemicals that

do not normally co-occur (Millero, 1986).

Vent and seep animals show a variety of behavioral and physiological adaptations to low and variable oxygen tensions. Behavioral mechanisms that help to maintain an aerobic metabolism in these environments include:

- spatially spanning an oxic/anoxic transition zone;
- temporally spanning the transition zone by moving from oxic to anoxic water;
- acquiring oxygen by pumping oxic water into an anoxic burrow or tube.

Alvinellids build tubes that protrude from chimney walls, allowing their gills access to oxygen-bearing ambient water. Mobile predators, such as *Bythograea therydron*, may move between pools of oxic and anoxic water. *Hesiocaeca methanicola*, a polychaete that can live on buried anoxic methane hydrates in the cold seeps of the Gulf of Mexico, appears to increase circulation of oxygenated water in its habitat using its parapodia (Fisher et al., 2000), as might the alvinellid polychaetes that live in tubes on hydrothermal chimneys (Desbruyères, 1998).

Many organisms found in hypoxic environments are able to maintain aerobic respiration and normal metabolic rate even at very low oxygen tensions (oxyregulation of respiration). A number of physiological adaptations are seen in oxyregulators, including large surface areas for gas exchange, short diffusion distances from external surface to blood spaces, a well-developed

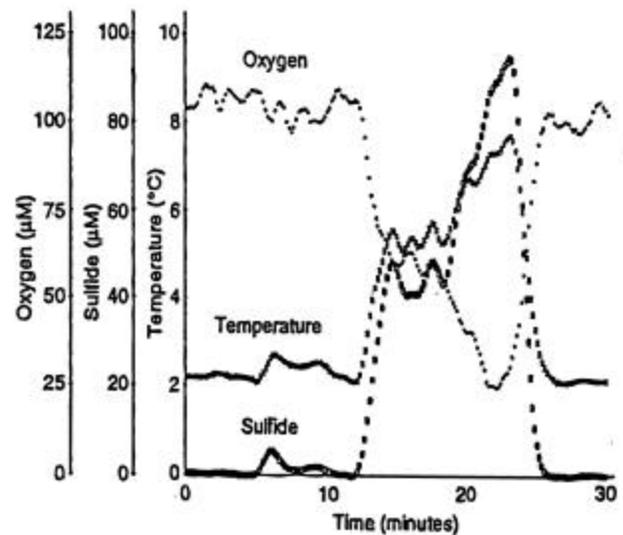


Figure 2. Temperature, Oxygen, and Sulfide Concentrations Measured in Situ at the Galapagos Rift. The sampling probe was moved from ambient bottom water to within a vent mussel bed and then back, a distance of about 50 cm. Oxygen and sulfide react spontaneously, and do not coexist for significant amounts of time. In areas where hot, sulfide-rich vent water mixes with cold, oxygen-rich ambient water, oxygen concentration decreases as water temperature and sulfide increase. (Johnson et al., 1986)

circulatory system, and the presence of respiratory pigments (Weber, 1978; Bryant, 1991). Some vent fauna, such as *B. thermydron*, have been shown to oxyregulate to very low levels of environmental oxygen (Mickel and Childress, 1982). A seep-endemic orbiiniid and the methane hydrate polychaete *H. methanicola* can also oxyregulate to very low oxygen tensions (Fisher et al., 2000; Hourdez et al., personal communication).

One common adaptation to improve oxygen exchange is the development of large gills or specialized surfaces for gas exchange, such as the plume of *R. pachyptila* (Jones, 1981, 1988; Arp et al., 1985) and the hypertrophied gills of Alvinellids (Jones, 1981; Jouin and Gaill, 1990). Decreased diffusion distances between gas exchange surfaces and the blood supply, another common adaptation that facilitates uptake of dissolved gases, has been documented in both alvinellids (Jouin and Gaill, 1990) and the seep orbiiniid (Hourdez et al., 2000). Finally, well-developed and highly vascularized circulatory systems have been found in alvinellids, vestimentiferans, and a seep orbiiniid (Jones, 1981, 1988; Jouin et al., 1996; Hourdez et al., 2000). Respiratory pigments, such as hemoglobin or hemocyanin, with high affinities and capacities for oxygen, are a particularly useful adaptation for an organism experiencing low and/or variable oxygen tensions. Although the respiratory pigments of most vent and seep fauna have not been characterized, both *Riftia pachyptila* and *Alvinella* spp. contain circulating hemoglobins with very high oxygen affinities (Terwilliger et al., 1980; Arp and Childress, 1981; Terwilliger and Terwilliger, 1984; Toulmond et al., 1990), which allows them to take up oxygen from very low concentrations and accumulate it to help withstand short periods of anoxia. *B. thermydron* hemocyanin affinity is increased by the presence of thiosulfate (a sulfide detoxification product) and lactate (a byproduct of anaerobic metabolism) (Sanders and Childress, 1992). *Riftia* hemoglobins (*R. pachyptila* has three different Hb's) bind sulfide, as well as oxygen, with high affinity and at high capacity, allowing simultaneous transport of both gasses to the symbionts in their internal trophosome while preventing the reaction of sulfide and oxygen in the blood (Arp and Childress, 1983; Childress et al., 1984). Alvinellid hemoglobin oxygen affinity is reduced by low pH (normal Bohr effect) and high temperature (Toulmond et al., 1990), which may facilitate the uptake and delivery of oxygen from the plume, normally extended outside of the tube in cooler waters, to the body, which is often bathed in highly reduced high-temperature fluids (Desbruyères et al, 1998; Cary et al., 1998).

Riftia hemoglobins have such a high affinity for oxygen that study of the binding properties is difficult, which has led to some variation among the results of different investigators. Overall, the data suggest that oxygen binding by *Riftia* hemoglobins shows a moderate normal Bohr and temperature effect that may assist offloading to the more posterior animal tissues, particularly in the trophosome,

where roughly two-thirds of the oxygen consumption occurs (reviewed in Childress and Fisher, 1992). The utility of the Bohr effect for oxygen offloading to the trophosome was questioned by Childress and Fisher (1992) because CO₂ consumption by the symbionts in the trophosome would overshadow tissue CO₂ production. However, Goffredi et al. (1999) have recently demonstrated a net H⁺ ion production by autotrophic *Riftia*, indicating H⁺ ion production in the trophosome, which could explain the utility of the Bohr effect in this symbiosis.

In the absence of oxygen, some organisms can use anaerobic metabolism for extended periods. Indeed, sulfide may poison aerobic respiration at the electron transport chain, forcing the organism to rely on anaerobic metabolism even in the presence of oxygen (Bryant, 1991). In general, annelids and molluscs are able to use more efficient mitochondrial pathways of fermentation (Bryant, 1991; Fenchel and Finlay, 1995; Tielens and Van Hellemond, 1998), whereas no crustacean has been found that can use a pathway beyond glycolysis, which yields only two to three ATP/glucose (reviewed in: DeZwaan and Putzer, 1985; and Bryant, 1991). Again, only selected vent and seep species have been tested for anaerobic tolerance. The vent crab *Bythograea thermydron* can survive only about 12 hours in the absence of oxygen, and the glycolytic endproduct lactate is accumulated during this time (Mickel and Childress, 1982). However, *Riftia* tolerates anoxia up to 60 hours and accumulates succinate when kept under anaerobic conditions, indicating the use of a modified citric acid cycle for fermentation (Arndt et al., 1998). Similarly, both the hydrate worm *Hesiocaeca methanicola* and a seep orbiiniid can survive four to five days in the absence of oxygen (Fisher et al., 2000; Hourdez et al., 2000).

Thus we see the same pattern with respect to oxygen that we saw with temperature. The vent and seep fauna that have been investigated are not significantly more tolerant of anoxia or high temperature than the best-adapted fauna from other environments, but are certainly very well adapted for extremes in these parameters.

TOXICITY

Potentially toxic chemicals abound in hydrothermal vent and cold seep environments (Corliss et al., 1979; Johnson et al., 1986; McDonald, 1990; Nix et al., 1995). Of these, sulfide is perhaps the most abundant and well studied, and its consequences for biological systems have been well documented in many other reducing environments, including mud flats, mangrove swamps, and sewage outfalls (see reviews in Somero et al., 1989 and Grieshaber and Volkel, 1998). Heavy metals may also occur in extremely high concentrations at hydrothermal vents, where they precipitate out of solution to form chimneys and sometimes coat tubeworm tubes and mollusk shells. In spite of our limited knowledge regarding specific adaptations to these toxins in vent and seep organisms, what we know of their physiologies and those of their shallower-

dwelling relatives should allow us to investigate some potential mechanisms for detoxifying these substances.

Sulfide

Sulfide is a toxin that, in just micromolar amounts, is capable of impairing biological processes necessary to metazoan function. Its most important physiological effect may be to severely inhibit aerobic respiration by interfering with cellular respiration and blood oxygen transport (reviewed in: Somero et al., 1989; Vismann, 1991; Grieshaber and Völkel, 1998). In the mitochondria, sulfide may poison the respiratory enzyme cytochrome *c* oxidase, thus inhibiting ATP production by the electron transport chain. Sulfide may also bind to the hemoglobin molecule in blood, reducing its capacity to carry oxygen and, in high concentrations, rendering it nonfunctional. In addition, a recent study found that sulfide is capable of inhibiting muscular contraction independent of its effects on aerobic metabolism (Julian et al., 1998).

To avoid these toxic effects, an organism has several practical options: avoid sulfide, switch to anaerobic metabolism, exclude sulfide from sensitive tissues, or oxidize sulfide to more benign forms. Most inhabitants of vent and seep environments do not realistically have the option of avoiding sulfide altogether. Species containing sulfide-oxidizing bacteria must supply this chemical to their symbionts, thus requiring them to inhabit areas where sulfide is abundant. Nonsymbiotic endemic heterotrophs not only must forage in areas where at least brief exposure is likely, but some must also consume symbiotic or free-living sulfide oxidizers that often contain high levels of sulfide (Somero et al., 1989). To prevent poisoning of the electron transport chain in the presence of high concentrations of sulfide, many invertebrates temporarily switch from aerobic to anaerobic metabolism. (Grieshaber and Völkel, 1998). As discussed above, several vent and seep species have considerable anaerobic capacity; whether this occurs in the presence of sulfide has not been directly tested. Exclusion from sensitive tissues and oxidation within the body are the two best-documented strategies to prevent sulfide poisoning among vent and seep animals, and symbiont-containing species often use them in conjunction.

Exclusion of sulfide from tissues may involve physical, biological, or chemical barriers around or within an animal. Thick tubes or cuticles may reduce or prevent exposure of some external tissues to sulfide, and epibiotic bacteria and abundant metal ions may oxidize sulfide before it makes contact with external tissues. The Pompeii worm, *Alvinella pompejana*, resides on active chimney structures where sulfide is abundant, within a secreted proteinaceous tube that it shares with epibiotic bacteria (Desbruyères et al., 1998). Although specific data is lacking, the tube is thought to provide a regulated environment (potentially lower in sulfide than the immediately surrounding vent fluid), and the bacteria to supply both nutrition and a means of sulfide detoxification for *A. pompejana* (Desbruyères et al., 1998). Several other worms living in direct contact with vent fluid, including the vestimentiferans, also secrete tubes and only expose portions of their bodies directly to sulfidic fluids. These structures may, in part, serve to limit what tissues and how much tissue surface area

are exposed to sulfide in the surrounding water.

Likewise, oxidation of sulfide to more benign sulfur compounds, most commonly thiosulfate, may occur within the animal by a variety of means, including sulfide-oxidase enzymes and mitochondrial oxidation. The vent crab *Bythograea thermydron* maintains aerobic metabolism by steadily increasing its rate of oxygen consumption up to environmental sulfide concentrations of about 800 μ M and apparently detoxifying sulfide via a sulfide-oxidase (Vetter et al., 1987; Childress and Fisher, 1992). Sulfide-oxidizing activity has also been found in tissues from several other vent species, including the crab *Munidopsis subsquamosa*, the shrimp *Alvinocaris lusca*, *Riftia pachyptila*, and *Calyptogenia magnifica* (Vetter et al., 1987; Powell and Somero, 1986b), as well as a host of species living in non-vent, reducing habitats (Lee et al., 1996; Grieshaber and Völkel, 1998). Oxidation of sulfide may also be coupled to energy production directly in the mitochondria of some animals or indirectly by providing reduced sulfur intermediates to symbionts. *Solemya reidi*, a clam that inhabits areas organically enriched by sewage and paper mill effluent, links sulfide oxidation to ATP production in its mitochondria at low-to-moderate sulfide concentrations, but this ability becomes inhibited at high concentrations (Powell and Somero, 1986a). Similarly, the intertidal lugworm *Arenicola marina* possesses the ability to oxidize sulfide in its mitochondria even at very high sulfide concentrations (Völkel and Grieshaber, 1996). It is not known whether sulfide oxidation by animal tissues of the vent mussel *Bathymodiolus thermophilus* is linked directly to ATP production, but the resultant thiosulfate is supplied to its bacterial endosymbionts, where it is further oxidized to fuel chemoautotrophic carbon fixation (Powell and Somero, 1986a; Fisher et al., 1987; Nelson and Fisher, 1995). In this way, sulfide oxidation in the animal's tissues is indirectly linked to energy production. As research continues at vents and seeps, sulfide oxidation mediated by mitochondria may prove to be a common method of detoxification.

Metazoans hosting sulfide-oxidizing bacterial symbionts must not only tolerate this potential toxin but must also acquire both sulfide and oxygen from the environment and transport them to the symbionts. In many cases, these animals employ specialized blood proteins that bind sulfide reversibly to prevent inhibition of oxygen transport, poisoning of cytochrome *c* oxidase in the animals' tissues, and spontaneous reaction of sulfide with oxygen. In *Riftia pachyptila*, two different extracellular hemoglobins in the vascular blood and one in the coelomic fluid bind sulfide and oxygen simultaneously and reversibly, with high affinity (Arp et al., 1985). Cysteine residues and disulfide groups on these hemoglobins apparently provide the sulfide-binding mechanism (Zal et al., 1998). Their presence as well on the extracellular hemoglobin of *A. pompejana* indicates that they may be a common adaptation to sulfide-rich vent habitats (Zal et al., 1997; Zal et al., 1998). These hemoglobins also bind sulfide with a high enough affinity to prevent the sulfide poisoning of cytochrome *c* oxidase (Powell and Somero, 1983). Deep-sea clams in the family Vesicomidae are also capable of binding both

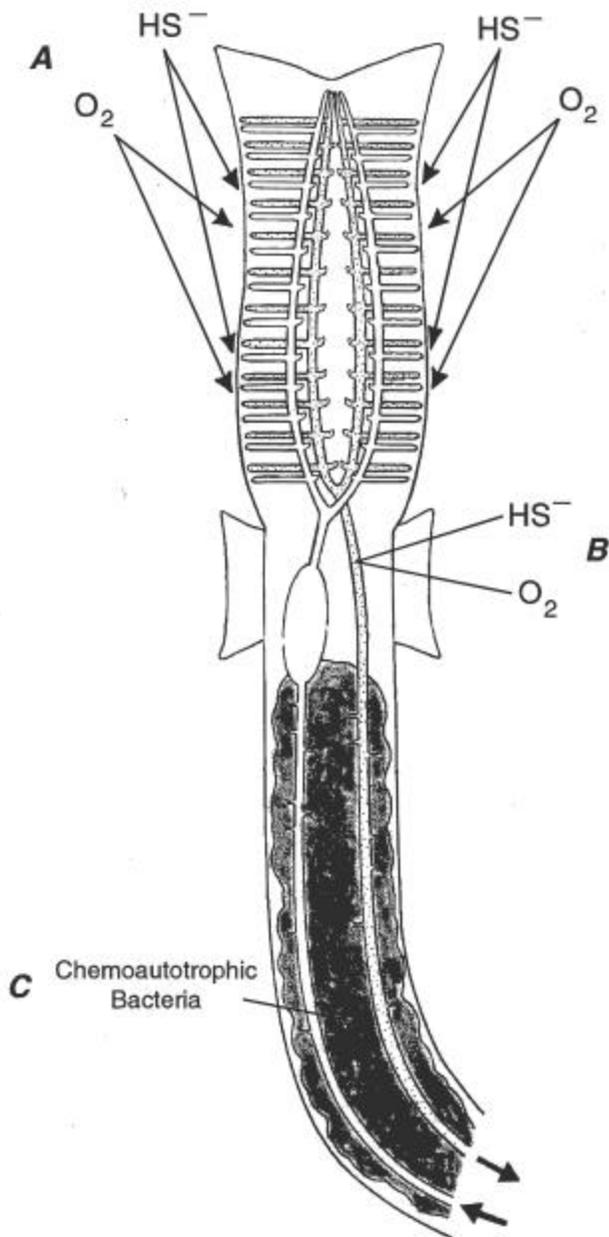


Figure 3. Mechanisms Allowing Vent Tubeworms to Tolerate and Exploit Their Sulfide-rich Environments. (A) Using its plume, the only part of its body that protrudes from the open end of the chitinous tube it inhabits, *Riftia pachyptila* acquires sulfide and oxygen from areas where vent fluids actively mix with ambient sea water. (B) Specialized hemoglobins in the blood of *R. pachyptila* simultaneously and reversibly bind both sulfide and oxygen, and (C) transport them to internally housed, sulfur-oxidizing, chemoautotrophic bacteria that act as a sink for the potentially poisonous sulfide. (Modified from Arp et al., 1985)

oxygen and sulfide simultaneously. However, unlike *R. pachyptila*, they utilize two different binding molecules: (1) an intracellular hemoglobin that binds oxygen with moderate affinity, and (2) an

extracellular sulfide binding factor that binds with high affinity (Arp et al., 1984; Childress et al., 1991). Sulfide binding can only protect sensitive tissues when a sink for the bound sulfide is available to remove sulfide and maintain free binding sites. In these species, internal chemoautotrophic microbial symbionts oxidize sulfide, acting as an internal sink while providing a source of fixed carbon for the host (Figure 3). All vestimentiferans and vesicomid clams appear to utilize a similar system to tolerate and exploit the sulfide-rich environments in which they live. This system is characterized by the exclusion of sulfide from sensitive tissues via high-affinity binding to blood components, transport to symbionts via the blood, and the oxidation of sulfide by intracellular chemoautotrophic bacteria.

Metals

Due to the interactions between circulating crustal water and hot basalts, dissolved heavy metals are particularly abundant in hydrothermal vent systems. Metals, in general, may interfere with a wide array of biological processes, including respiration, muscular function, osmoregulation, reproduction, development, and protein utilization (Luoma and Carter, 1991). Metals can also cause morphological abnormalities, histopathological problems, and instability of genetic material (Luoma and Carter, 1991).

Metazoans typically detoxify absorbed or ingested metals by using metal-binding proteins (metallothioneins) and forming subcellular inclusions. These mechanisms often act jointly to consolidate and enclose excess metals, which then accumulate within tissues and/or skeletal structures over time (Beeby, 1991; Luoma and Carter, 1991). The few investigations into potential metal detoxification in vent and seep fauna indicate that strategies used by these animals are not very different from those studied elsewhere. Polychaetes of the genus *Paralvinella* secrete mucus, rich in metallothionein-like proteins, that removes inorganic material from the epidermis and may also remove metals from the immediate external environment (Juniper et al., 1986). Metallothionein-like proteins and metal-rich inclusions have been found within the tissues of *R. pachyptila*, *A. pompejana*, *C. magnifica*, and a Bathymodiolid mussel from the Mid-Atlantic Ridge, and elevated levels of metals have been found within the shells of *C. magnifica* and *B. thermophilus* (reviewed in Childress and Fisher, 1992; Geret et al., 1998). What may separate vent and seep species from shallower marine taxa is not the detoxification mechanism, per se, but rather the ability of the mechanism to function effectively at high metal concentrations and over long exposure times.

SUMMARY AND CONCLUSIONS

Metazoans colonizing vent and seep habitats must tolerate not only the already extreme characteristics of the deep sea but also a wide range of additional conditions that result from the complex geologic and microbiological processes driving these environments. Ironically, high concentrations of hydrogen sulfide, one of the primary characteristics that should make these environments inhospitable to metazoan life, also drives biological production to

levels far exceeding those of the surrounding deep sea. To exploit the energetic abundance of vents and seeps, metazoans must tolerate not only the sulfide but also a whole suite of factors intrinsically correlated with its presence. At vents, the presence of sulfide corresponds directly to the high temperatures, absence of oxygen, and presence of heavy metals characteristic of the effluent waters. At seeps, as at vents, the presence of sulfide corresponds to the absence of oxygen; but, unlike the situation at vents, it also corresponds to the presence of potentially toxic hydrocarbons. Although not well studied in seep fauna, crude oil and its individual chemical components display fouling and mutagenic effects on a host of biological functions, including feeding, respiration, excretion, reproduction, development, and chemoreception (Bayne et al., 1982; Suchanek, 1993).

To cope with the numerous, potentially interacting extremes of these environments, the denizens of vents and seeps employ and combine a vast array of morphological, physiological, and behavioral adaptations. By inhabiting active chimney structures at hydrothermal vents, *A. pompejana* and *P. sulfinicola* experience extremes in temperature, hypoxia, sulfide, and heavy metals. They tolerate such varied extremes by modifying their microenvironments, interacting with other organisms, and relying on specialized biochemical adaptations. Combined, such adaptations allow these species to exploit an environment where very few other metazoans could survive.

Much of what we know regarding metazoan adaptations to these deep-sea reducing environments comes from investigations at hydrothermal vents. Therefore, research into other extreme deep-sea environments is sure to expose previously unknown and novel adaptations. At cold seep sites on the upper Louisiana slope in the Gulf of Mexico, for example, methane hydrates and brine pools, like chimney structures at hydrothermal vents, present combinations of extreme environmental characteristics, yet they support surprisingly dense metazoan communities. Although methane hydrates are characterized by the presence of sulfide, methane, and crude oil, and a corresponding absence of oxygen, the polychaete *H. methanicola* flourishes on their surfaces (Fisher et al., 2000). Similarly, the brine pools in this region possess salt concentrations several times higher than normal seawater, almost no oxygen, and abundant crude oil; nonetheless, massive communities of methanotrophic mussels sometimes live on their surfaces (MacDonald et al., 1990).

The low diversity of species found at hydrothermal vents and cold seeps, as compared to other deep-sea environments, clearly suggests that few organisms have been able to successfully establish and reproduce under the extreme conditions these habitats present. Yet the high density, high standing-stock biomass, and high levels of production in the communities that do persist in these environments indicate a significant advantage to those organisms capable of tolerating these conditions. By tolerating extreme environments, these select few animals take advantage of an abundant yet toxic energy source, which allows them to flourish in the very nutrient-poor deep sea.

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