Temperature and sulphide tolerance of hydrothermal vent fauna

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Hydrothermal vent communities have very high biomass, which is dominated by relatively few endemic species. Considering the stark contrast between the amount of organic material available at the vents to that in most of the surrounding deep-sea, one might expect that more of the ambient deep-sea fauna would colonize these environments. However, the toxicity of the hydrothermal fluids and the variability of the chemical and thermal regime the vent fauna experience require specific adaptations before an organism can survive there. The aspects of the environment which are most different from that of the surrounding deep-sea are those induced by the spatially and temporally variable input of hydrothermal fluid into the environment, which results in habitats where temperature, pH, and chemical content (including dissolved gasses) vary greatly over short times and distances. At the highest levels of exposure to vent fluids where metazoans have been documented, the water is anoxic, laden with sulphide and heavy metals, and the average temperature is at least 35°C. In some habitats it is likely that the animals are occasionally in brief contact with water in the 100°C range. Overlaying this extreme, and mixing with it to create a range of intermediate chemical and physical conditions, is the cold, oxic and relatively pristine ambient deep-sea water. The hydrothermal vent fauna have not only adapted to live in these conditions, but some of the species are among the fastest growing invertebrates on the planet.

To survive in the hydrothermal vent environment, the fauna have adaptations which allow exposure to a range of temperatures, while maintaining a supply of oxygen to their tissues and avoiding the toxic effects of their environment. Simple exclusion of the dissolved toxins would seem to be untenable when one considers the fact that these animals are aerobic and must utilise respiratory surfaces for gas exchange. This is especially true for the chemoautotrophic symbiont-containing animals, because they have the added requirement of supplying reduced sulphur compounds, usually the toxic gas hydrogen sulphide, to their symbionts. Understanding how the vent fauna tolerate and thrive in this hostile environment has been the focus of a majority of the physiological investigations of these animals. In parallel with these studies, biologists have long recognized the importance of characterizing the microhabitats occupied by the vent fauna. However, we have also recognized the difficulty in doing so because not only are the microhabitats temporally variable, but some of the spatial gradients of temperature and dissolved gasses are arguably the greatest of any habitat on the planet. For example, temperature gradients of well over 100°C per cm are not uncommon in chimney environments, and can exceed 300°C per cm on flanges.

A review of some thermal tolerance investigations of vent fauna provides an excellent overview of the variety of approaches available to deep-sea physiological ecologists interested in such questions. Terrestrial and shallow water biologists have the luxury of working both with animals which are relatively easy to maintain alive and with systems amenable to technologically complex field studies. One approach often used is to determine the temperature inducing 50% mortality (LT_{50}) for a species. This measurement can establish the upper physiological limitations with respect to body temperatures and times of exposure, although the experimental design often limits behavioural responses of the test organism. Results of these types of experiments emphasize the importance of time of exposure. For example the LT_{50} for a typical temperate shallow-water starfish is 42°C with 9 min. exposure, but only 32°C with 40 min. exposure. The much more elegant experiments with fine gauge thermocouples inserted into live animals released in their natural environment have

provided definitive data on body temperatures experienced by animals living in high temperature habitats. For example some desert bees can fly with sustained body temperatures as high as 46°C and some desert ants regularly survive body temperatures of up to 55°C for short periods in their natural environment. To date neither LT_{50} experiments nor in situ body temperature measurements have been made with vent fauna, so information on this subject is limited to attempts to characterize their microhabitat, studies on isolated tissues, organelles and molecules, and in one case experiments on live animals in pressure aquaria.

In the case of the hydrothermal vent crab *Bythograea thermydron* Williams, 1980, the types of tissue thermal tolerance measurements, which have been made on many vent invertebrates, were supplemented with physiological measurements on living animals in high pressure aquaria. E. Dahlhoff and G. Somero measured the K_M values of NADH for malate dehydrogenase of several vent and non-vent species at habitat pressure and 5°C. Regression of K_M values against habitat temperature for animals from well characterized thermal habitats yielded a high r^2 and this relation was used to infer the thermal tolerance of several species of vent animals from the measured K_M values. They concluded that *B. thermydron* could withstand a maximum sustained body temperature of about 25°C from these data. In another study by the same lab, Dahlhoff and co-workers determined the Arrhenius break temperature of mitochondrial respiration (the temperature at which respiration ceases to increase with increasing temperature) for a variety of vent and non-vent animals. They found that *B. thermydron* had a break temperature of 46°C, quite close to those of the East Pacific Rise (EPR) alvinellids and *Riftia pachyptila* Jones, 1981 and concluded that all experienced similar maximal body temperatures in the 25 - 40°C range. Studies by A. Arp and co-workers on the isolated haemocyanin of the crab indicate it is functional to at least 30°C. T. Mickel and J. Childress found 100% mortality in animals exposed to 37.5°C for one hour at their habitat pressure and 100% survival in animals exposed to 35°C. However they found significant perturbations in the heartbeat of animals exposed to 30°C. Taken together these data indicate that the crabs can tolerate sustained exposure to temperatures of about 25°C and short exposures to temperatures in the 30 - 35°C range. The concordance of the direct measurements on living crabs with the conclusions reached from the studies of isolated biomolecules and mitochondria suggests that although there are certainly caveats to applying results from studies of isolated tissues and molecules to live animals, the approach is valid for approximations of thermal tolerance.

The vent animals which inhabit environments with the steepest temperature gradients and potential exposure to the highest temperatures, are some of the chimney fauna such as the alvinellid polychaetes *Paralvinella sulfincola* Desbruyères & Laubier, 1993 in the NE pacific, and *Alvinella pompejana* Desbruyères & Laubier, 1980 and *A. caudata* Desbruyères & Laubier, 1986 on the EPR. Because the gradient is so extreme, obtaining appropriate in situ temperature measurements has been very difficult, and even with very fine scale spatial and temporal control of the measurements one can never be sure that behaviour and currents created by the living animals do not dramatically affect the actual body temperature experienced by the worms. The classic digital image of an EPR alvinellid curled around the end of a temperature probe which is registering 105°C fired the imagination of many biologists and a variety of physiological investigations have addressed the temperature tolerance of these species. Unfortunately these polychaetes have never been maintained satisfactorily on board ship for studies with live animals, so our only direct information of their temperature tolerance comes from studies of isolated tissues, organelles and biomolecules.

From measurements of the K_M of NADH for malate dehydrogenase (like those described above), the same investigators concluded that the EPR alvinellids could withstand a maximum sustained body temperature of about 31°C. Dahlhoff and co-workers also determined that the Arrhenius break temperature of mitochondrial respiration for these worms was similar to that of *B. thermydron* and concluded that their habitat temperatures were similar. Studies by Toulmond and co-workers on the haemoglobins of this group found that the binding properties were most stable between 20 - 30°C, suggesting that this was their normal body temperature, and that the haemoglobins broke down at 50°C, suggesting that their critical maximal temperature would not exceed this. Studies by F. Gaill and co-workers have demonstrated that the cuticle and interstitial collagens of the EPR alvinellids are stable at higher temperatures than those of other annelids, but that these collagens melt between 44 - 46°C. Taken together these data suggest that the EPR alvinellids can withstand body temperatures of about 30 - 35°C for extended periods, but that allowing their body temperature to rise to 50°C would be lethal.

This conclusion seems to be in conflict with the substantially higher in situ habitat temperatures measured by numerous different investigators over the years. However, it should be kept in mind that the body temperatures of alvinellids in situ have not been measured, and it is likely that their behaviour will have a substantial effect on their immediate thermal environment and their resultant body temperature. Thus, although alvinellid worms are undoubtedly living in close association to one of the hottest environments on the planet colonized by metazoans, and are perhaps surviving brief external

exposure to higher temperatures than other metazoans, their physiological thermotolerance is most likely similar to that of other high temperature adapted invertebrates.

The toxicity of the vent fluid is another aspect of the vent environment requiring special adaptations in the fauna. Interestingly, one of the most potentially toxic molecules which occurs in high concentrations in vent fluids, hydrogen sulphide, is also the most important source of energy for the chemoautotrophic production which fuels the hydrothermal vent communities. Hydrogen sulphide is toxic to many animals in µM concentrations due to its effect on mitochondria. Hydrothermal vent animals are commonly exposed to concentrations in excess of 100 µM in dilute vent fluids. Conceptually, there are a variety of ways the vent fauna could adapt to exposure to high levels of sulphide. One would be to simply avoid exposure to sulphide. Behavioural adaptations in the most mobile fauna can minimise the exposure somewhat. However, all the vent animals must obtain oxygen across their respiratory surfaces, and moderate to high levels of exposure to sulphide is inevitable for most vent fauna. Theoretically, evolution of a sulphide insensitive cytochrome c oxidase would be viable, but M. Powell and G. Somero found no such adaptations in the vent animals they analysed. An approach which is fairly common among the fauna which inhabit reduced and anaerobic sediments in shallow water, is an increase in anaerobic capacity. This has not been systematically investigated in vent fauna, and will likely prove to be true in at least some vent species. The most common defence against sulphide appears to be enzymatic detoxification to thiosulfate. This has been demonstrated in all of the vent fauna tested to date. M. Powell and G. Somero also found that *Solemya reidi* Bernard, 1980, a clam which inhabits sewage and paper mill effluent sites on the west coast of the United States, could couple the oxidation of sulphide to ATP production, thus providing energy to the animal. This ability may also be common in vent animals as well as other metazoans which occupy sulfidic habitats.

A variety of the hydrothermal vent fauna have evolved symbiotic relationships with sulphur oxidizing chemoautotrophic bacteria. In most of these associations it is well accepted that the symbionts are the primary source of nutrition for their hosts, and therefore exposure to the sulphide rich vent effluent is required for the health of these animals. When the symbionts are external (such as on the Alvinellids of the EPR or the shrimp of the Mid Atlantic Ridge), the symbionts may help minimize exposure of the animal tissues to sulphide by consuming it externally, and there are no additional demands on the animal to tolerate or transport sulphide internally. Thus, although maintenance of the symbiosis requires exposure to sulphide, the symbionts themselves may assist in protecting the animal from the molecule. The vent mussels with intracellular chemoautotrophic symbionts apparently detoxify sulphide to thiosulfate, like most of the non-symbiont containing fauna, and then supply thiosulfate to their symbionts. Although thiosulfate is not as reduced an energy source as sulphide, this approach provides an adequate supply of energy to the symbionts without extensive unique biochemical adaptations in the host. However, in the case of vestimentiferan tubeworms, the symbionts are intracellular in an internal organ, and require hydrogen sulphide as an energy source. To meet this need without poisoning their own tissues the vestimentiferans have evolved special haemoglobins which bind sulphide with very high affinity. Their deep red blood has a high capacity for sulphide and transports mM concentrations of sulphide through their body to their symbionts. Symbiont sulphide consumption provides the sink necessary to keep the blood from becoming saturated with sulphide while continuously acquiring it from the environment. Since the sulphide is tightly bound in the blood, the animals tissues are protected from its toxic effects. A positive side effect of this method of avoiding the toxic effects of sulphide is that it may render the tube-worm's tissues unpalatable or even poisonous to most animals. The mM levels of sulphide in vestimentiferan blood are released upon acidification such as would happen in a consumer's gut. This may in part explain the apparent lack of predation on the worms by non-vent fauna. However, one hydrothermal vent top consumer, *Bythograea thermydron,* has high levels of sulphide oxidase in its hepatopancrease and has often been observed picking at the exposed, blood-filled, tentacular plumes of the vent tube-worms.

The characteristics of the vent environment which make it so inhospitable to most metazoans also contribute to the success of those species adapted to it. Not only do many of the species grow very quickly and/or reach high densities, but the vent communities seem to be relatively free of predation from the ambient deep sea fauna. Perhaps the old expression, "if you can't take the heat, stay out of the kitchen" should be augmented with "but if you can it's a great place to be".

Acknowledgements

References

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