THE ROYAL SOCIETY

doi 10.1098/rspb.2000.1286

Life in the extreme environment at a hydrothermal vent: haemoglobin in a deep-sea copepod

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This is the first study, to my knowledge, quantifying the respiratory pigment haemoglobin discovered in a deep-sea copepod. Haemoglobin in copepods has previously been documented in only one other species from the deep water of an Italian lake. Specimens of the siphonostomatoid *Scotoecetes introrsus* Humes were collected during submersible dives at 2500 m depth near a hydrothermal vent at the East Pacific Rise (9° N). The haemoglobin content in the copepods' haemolymph was 4.3 ± 0.6 μg per individual female ($n = 6$) and 1.8 ± 0.1 μg per individual male ($n = 6$). Weight-specific concentrations of haemoglobin were identical for females and males (0.25 ± 0.04 and 0.26 ± 0.02 μg per microgram dry weight, respectively). These haemoglobin concentrations are higher than those found in other small crustaceans. Activity of the electron transport system indicated that the respiration rates in *S. introrsus* (13.7 ± 7.7 μl O_2 per milligram dry weight per hour) were similar to those in the shallow-water copepod *Acartia tonsa* (9.1 ± 1.3 μl O_2 per milligram dry weight per hour). It was concluded that the possession of highly concentrated haemoglobin allows *S. introrsus* to colonize a geologically young, thermally active site such as the vicinity of a hydrothermal vent, despite the prevailing oxygen depletion.

Keywords: haemoglobin; respiration; oxygen depletion; copepod; deep sea

1. INTRODUCTION

Copepods are ubiquitous crustaceans living in a broad variety of aquatic habitats, most of them having in common the characteristic of providing non-limiting concentrations of oxygen dissolved in the water. Extreme physico-chemical conditions may form special habitats, such as the oxygen-depleted waters in the vicinity of deep-sea hydrothermal vents. Expelling hot (up to 350 °C), mineral-rich and anoxic fluid, vents create turbulence and pronounced small-scale variations in environmental conditions with strong gradients toward the surrounding cold (2 °C) and oxygen-rich deep-sea water. Organisms living in the vicinity of the vents might take advantage of elevated temperatures, nutrient concentrations and growth rates of bacteria or other organisms serving as prey. At the same time, they are temporarily or permanently exposed to oxygen deficiency.

As an adaptation to oxygen deficiency, a variety of taxonomically unrelated organisms in vent habitats possess respiratory pigments that enhance their affinity to oxygen and allow survival at lower oxygen concentrations. Haemocyanin is found in brachyuran crabs (Arp & Childress 1981*b*) and haemoglobin in the vestimentiferan tubeworm *Riftia pachyptila* (Arp & Childress 1981*a*; Wittenberg *et al.* 1981), in polychaetes *Alvinella* spp. and *Paralvinella* spp. (Terwilliger & Terwilliger 1984; Toulmond *et al.* 1990; Jouin-Toulmond *et al.* 1996) and in the clam *Calyptogena magnifica* (Terwilliger *et al.* 1983). Despite the vast number of copepod species known, including many that are endemic to hydrothermal vents (e.g. Humes 1987, 1989), respiratory pigments in copepods have so far been unknown. The only exception is one record of haemoglobin in the copepod *Attheyella crassa* (Sars) from the bottom of Lago Maggiore, Italy, which was found at a depth of 120 m (Fox 1957).

Haemoglobin might allow hydrothermal vent copepods to survive under adverse conditions in places offering little oxygen, unlike habitats in shallow water or in deep water at off-vent sites. Similar to surface water, which is ordinarily without hydrogen sulphide and contains oxygen concentrations near saturation, ambient deep-sea water at 2500 m in the eastern Pacific contains oxygen concentrations of *ca.* 110 μM and no hydrogen sulphide (Johnson *et al.* 1988; Childress & Fisher 1992). Sulphide concentrations generally increase with increasing temperatures from the ambient deep sea towards a hydrothermal vent, whereas oxygen concentrations decrease (Johnson *et al.* 1986). Oxygen concentrations in the vent water itself are reduced to zero. Johnson *et al.* (1986) measured between 70 and 25 μM oxygen and 45–95 μM sulphide in water of 5–8 °C. Communities in close vicinity to vents with the polychaete *Paralvinella* commonly experience even lower oxygen concentrations and sulphide of up to 100–300 μM (Juniper & Martineu 1995; Sarrazin *et al.* 1999).

In the current study, I measured concentrations of the respiratory pigment haemoglobin, which was discovered in a copepod living in association with *Paralvinella* at a hydrothermal vent on the East Pacific Rise. I also estimated the copepod's *in situ* respiration rates from measurements of the activity of its electron transport system (ETS) and compared those to respiration rates found in shallow-water copepods that are not exposed to low oxygen conditions.

2. MATERIAL AND METHODS

Specimens of the siphonostomatoid copepod *Scotoecetes introrsus* Humes (1987) were collected at the East Pacific Rise (9° 50.8′ N, 104° 17.6′ W) during two dives with the submersible 'Alvin'. At 2500 m depth within 2–3 m distance of an active

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Received 28 June 2000 *Accepted* 21 August 2000

vent ('Q-Vent') the copepods lived in association with the vent-endemic polychaete *Paralvinella grasslei* (Desbruyères & Laubier 1982) within a clump of the vestimentiferan tubeworm *R. pachyptila*.

Temperatures were not measured simultaneously with the sampling, but at a long-term mooring placed in a similar association of haemoglobin-containing copepods with *Paralvinella* and *Riftia*. The temperature varied between 15 and 25 °C over periods of weeks to months, with an exceptional decrease to 10 °C and fluctuated over only a few degrees within a day (H. Hunt, unpublished data).

After retrieval of the submersible, the copepods were stored in a cold room (3 °C) or on ice until sample preparation was finished, during which individual copepods were manually cleared from possible attachments of *Paralvinella* mucus or detritus. The copepods were frozen at − 70 °C within 12 h of the time of catch in separate samples of adult females and males. Subsequent haemoglobin analyses were performed with a photometric assay according to Landon & Stasiak (1983). One hundred microlitres of aqua dest were added to samples of 50 individuals each, the samples sonified on ice and aqua dest added to a total volume of 1.5 ml. After 5 min centrifugation at 10 000 *g* and 4 °C, potassium cyanide solution was added to the cell-free supernatant in order to prevent deoxygenation of oxyhaemoglobin. The wavelength for the analyses was 418.5 nm (the absorption peak for both copepod haemoglobin and a standard of double-crystallized rabbit haemoglobin (SIGMA)).

In order to estimate respiration rates, ETS activity was measured in eight samples of 22–50 individuals each that were all alive when sorted aboard ship (assay temperature 15 ± 1 °C, for the protocol see Packard & Williams (1981) and Dortch *et al.* (1996)). The enzyme assay quantifies respiration potential by measuring oxidative phosphorylation. It uses an artificial electron acceptor (INT) and a substrate solution containing NADH and NADPH for quantifying colour development as INT is reduced to formazan.

The body lengths of the copepods were measured in subsets of the samples for the haemoglobin and ETS analyses. The cephalothorax lengths of the copepods were 0.70 ± 0.05 mm ($n = 73$) for males and 1.06 ± 0.06 mm ($n = 161$) for females. Their total body sizes, caudal rami excluded, were 1.33 ± 0.08 mm ($n = 17$) and 1.72 ± 0.11 mm ($n = 44$), respectively. Their dry weights were determined as 17 ± 1 μg ($n = 3$) for females and 7 ± 1 μg ($n = 2$) for males in pooled samples of eight copepods each of which was dried at 65 °C overnight and weighed on a Mettler MT5 balance.

Individuals of the marine, shallow-water copepod *Acartia tonsa* were kept in well-aerated laboratory cultures for the comparative ETS assays and then analysed in five samples of 50 adults each. An average body weight of 10 μg per individual (Kiørboe *et al.* 1985; Tiselius 1998) was used for calculation of weight-specific rates.

For species determination of the deep-sea copepod preserved individuals were embedded in lactic acid (Humes 1964) and identified (Humes 1987, 1989).

3. RESULTS

The haemolymph of *S. introrsus* showed an absorption spectrum typical of haemoglobin (figure 1). The total amount of haemoglobin in the haemolymph of an individual copepod was 1.82 ± 0.14 μg per individual for males and 4.33 ± 0.63 μg per individual for females (table 1).

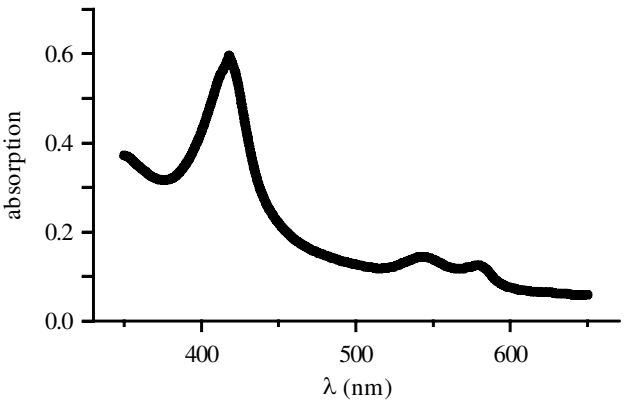

Figure 1. Absorption spectrum of the oxygenated haemoglobin of *S. introrsus*. The absorption maxima were 579 nm with range 578–580 nm for α absorption ($n = 12$), 544 nm with range 540–545 nm for β absorption and 418.5 nm for the main (Soret) peak (same in all samples). The ratio of α:β absorption was 0.86 ± 0.02).

Table 1. *Haemoglobin content per individual copepod and concentration of haemoglobin in the haemolymph*

	Alvin dive number					
	AD 3311		AD 3317		mean (s.d.)	
	male	female	male	female	male	female
haemoglobin	2.02	4.98	1.75	3.76	—	—
(microgram per	1.72	4.59	1.65	3.92	—	—
individual)	1.90	5.08	1.86	3.66	1.82 (0.14)	4.33 (0.63)
haemoglobin	0.29	0.29	0.25	0.22	—	—
(microgram per	0.25	0.27	0.24	0.23	—	—
microgram						
dry weight)	0.27	0.30	0.27	0.22	0.26 (0.02)	0.25 (0.04)

This difference between genders was merely due to body size. The concentration of haemoglobin in the haemolymph was not significantly different (*t*-test, $t = 0.265$ and $p = 0.399$), with 0.26 ± 0.02 μg per microgram dry weight for males and 0.25 ± 0.04 μg per microgram dry weight for females.

The ETS activity of *S. introrsus* was equivalent to an individual rate of 0.173 ± 0.054 μl O_2 per individual per hour or a weight-specific rate of 13.7 ± 7.7 μl O_2 per milligram dry weight per hour. The individual-based ETS activity in *A. tonsa* was 0.09 ± 0.01 μl O_2 per individual per hour, which is equivalent to 9.1 ± 1.3 μl O_2 per milligram dry weight per hour. The difference in weight-specific ETS activities between the deep-sea copepod ($n = 8$) and the shallow-water copepod ($n = 5$) was non-significant (*t*-test, $t = 1.647$ and $p = 0.069$).

4. DISCUSSION

Scotoecetes introrsus was found attached to the mucus covering the alvinellid *P. grasslei* which was growing within a clump of the tubeworm *R. pachyptila*. Alvinellid

polychaetes are among the first metazoans to have colonized newly formed sulphide vent chimneys (Juniper & Martineu 1995). Living in association with these, *S. introrsus* might be a pioneer among crustaceans as it is able to settle in a geologically young, thermally active environment. Very hot, anoxic vent water (up to 350 °C) mixes with cold, oxygen-rich, ambient deep-sea water (2 °C) in alvinellid habitats in proximity to active vents. *P. grasslei* lives on the colder end of this sharp temperature gradient but is exposed to high-frequency changes in temperature and partial pressure of oxygen (Johnson *et al*. 1986; Toulmond *et al*. 1990). The temperatures *Riftia* normally encounter fluctuate between 2 and 23 °C depending on the current field generated by the thermal activity of the vent (Wittenberg *et al*. 1981) and the oxygen concentrations that these sessile organisms are exposed to change simultaneously. The copepod *S. introrsus* is likely to experience similar temperature and oxygen conditions.

Haemoglobin enhances the haemolymph's affinity for oxygen dissolved in the surrounding water, often more than other respiratory pigments. Haemocyanin in the blood of the hydrothermal vent brachyuran crab *Bythograea thermydron* has only moderate oxygen affinity (Arp & Childress 1981*b*). Several phylogenetically unrelated organisms inhabiting the vicinity of active hydrothermal vents produce haemoglobin as a respiratory pigment (Childress & Fisher 1992). In contrast to the crabs, many of these are sessile, such as the tubeworm *R. pachyptila*, the clam *C. magnifica* and the polychaetes *Alvinella* spp. and *Paralvinella* spp., and unable to move to regions of higher oxygen concentations temporarily. Due to its attachment to the worms, the copepod *S. introrsus* is also sessile, unlike many other copepods.

The concentration of haemoglobin found in *S. introrsus* is considerably higher than that found in other small crustaceans. Maximal haemoglobin concentrations of 0.12 μg (Sell 1998) and 0.155 μg (Landon & Stasiak 1983) per microgram dry weight have been reported for the freshwater cladoceran *Daphnia pulex* when living under continuous oxygen deficiency. This is only *ca*. 50% of the concentration measured in the deep-sea copepod.

Kobayashi & Hoshi (1984) observed that individual *Daphnia* with high concentrations of haemoglobin were able to maintain normal oxygen consumption rates, even at oxygen concentrations as low as those that are lethal for individuals lacking haemoglobin. The 'normal' respiration rates of *S. introrsus* found here suggest that it may have the same capability.

The blood of various vent animals has the ability to detoxify sulphide entering from low-oxygen vent water (Powell & Somero 1986). It has not yet been investigated whether the blood of *S. introrsus* has this ability. Sulphide binding in the blood has not yet been shown experimentally for *Paralvinella* spp. either (Juniper & Martineu 1995), but Jouin-Toulmond *et al.* (1996) suggested that the polychaetes might be able to detoxify sulphide entering the blood through the production of haematin, which is capable of sulphide detoxification (Powell & Arp 1989). Alternative suggestions for mechanisms protecting the worm tissues from sulphide include colonization by sulphide-oxidizing bacteria or frequent shedding of mineral-accumulating epidermal mucus (Juniper & Martineu 1995). *S. introrsus* live in the mineral-enriched mucus of *Paralvinella* and are presumably exposed to very high concentrations of external sulphide, therefore making the use of a mechanism of sulphide detoxification likely.

Measurements of enzymatic activity give useful estimates of the respiration rates of shallow- or deep-living marine animals (Childress & Somero 1979). The long latency of the ETS response to a change in the external environment allows for quantification of *in situ* respiration (Båmstedt 1980; Mayzaud 1986). The deep-sea copepod *S. introrsus* and the shallow-water copepod *A. tonsa* had similar respiration rates. Childress *et al*. (1990) confirmed that oxygen consumption rates in deep-sea crustaceans were not influenced by depth, i.e. pressure, but depended primarily on temperature. The analyses in this study, which were performed at identical temperatures, should be directly comparable. Hand & Somero (1983) measured the activities of enzymes for energy metabolism in several hydrothermal vent organisms and found that, in general, their activities were similar to those of related, shallow-living marine species. Several of the animals investigated in their study did possess haemoglobin. Analogously, Hourdez *et al*. (1999) found haemoglobin in deep-sea scaleworms but not in littoral members of the same family (Polychaeta: Polynoidae). I conclude that the presence of haemoglobin in *S. introrsus* allows this vent copepod to maintain 'normal' respiration rates in an environment with highly variable and often low concentrations of dissolved oxygen.

Many thanks go to Lauren Mullineaux and Donal Manahan for providing me with enthusiasm, ship time and the opportunity for diving to the hydrothermal vents. I also thank the crew of R/V 'Atlantis' and 'Alvin' for their support, Jim Ruzicka for help with the ETS analyses, Don Anderson and Dave Kulis for space and help in the laboratory and Anna Metaxas, Peter Tiselius and two anonymous reviewers for critical comments on the manuscript. This work was supported by US National Science Foundation grant OCE-9619605 to Lauren Mullineaux, postdoctoral funding by the Seaver Foundation and through a Royal Swedish Academy of Sciences & Munkedals AB fellowship to A.S. and travel support through a Pew Fellowship in Conservation and the Environment granted to Judy McDowell. This is WHOI contribution number 10220.

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