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Metabolic rate of the brackish water polychaete *Marenzelleria viridis* under reducing conditions[1]

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Abstract

Hydrogen sulphide is known to be a potent inhibitor of the respiratory chain and leads to a depression of aerobic metabolism. Organisms living in sulphidic habitats such as shallow coastal water inlets have to cope with its toxic effects. In the Southern Baltic the species *Marenzelleria viridis*, native to brackish water habitats in North-East America has proliferated rapidly and become a characteristic faunal element. The polytrophic conditions cause a high concentration of sulphide in the sediment, where the animals inhabit J-shaped burrows. Since H_2S is thought to depress aerobic metabolism, the metabolic rate of the animal was investigated by means of direct and indirect calorimetry. The heat production of adults was significantly enhanced when exposed to sulphide (250 μmol l^{-1}) and hypoxic conditions. In addition larvae of *M. viridis* increased their metabolic rate in the presence of sulphide if oxygen supply was sufficient. In the absence of oxygen the heat dissipation was only slightly raised reflecting different detoxification processes in the presence and absence of oxygen. The enhanced metabolic rate at hypoxic, sulfidic conditions may be due to detoxification processes which are coupled with an energy gaining system used by the animal itself or which may be associated with bacteria.

Keywords: Direct calorimetry; Hydrogen sulphide; *Marenzelleria viridis*; Metabolic rate; Polychaete

1. Introduction

Marenzelleria viridis, a polychaete species native to the eastern coast of North America, has recently been reported from several brackish water habitats of Northern Europe [1,2]. *M. viridis* appeared in the coastal inlets (Darss-Zingster Bodden chain) of the

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Southern Baltic during the mid-1980s [3], becoming the characteristic faunal element throughout the 1990s with an abundance of 8000 adults per m^2 recorded in 1992 [4].

The Darss-Zingster Bodden chain is a shallow biotope with a salinity gradient from freshwater to about 12 ppt and a trophy gradient from eutrophic to polytrophic conditions [5]. This results in the periodic occurrence of hypoxic or anoxic regimes. The trophy of the shallow coastal inlet leads also to an increasing content of hydrogen sulphide in the sediments. H_2S is mainly produced by bacterial sulphate reduction or by microbial degradation of organic material; both processes are restricted to anaerobic processes in the sediment under reducing conditions [6]. Hydrogen sulphide is known to be a potent inhibitor of several enzymes and of the cytochrome *c* oxidase thus causing a breakdown of aerobic respiration at low nanomolar concentrations [7, 8]. *M. viridis* inhabits 30 cm long J-shaped burrows in the sulfidic smelling sediment of the bodden chain and has to cope with daily and annually fluctuating sulphide and low oxygen concentration.

The rapid and successful propagation of this polychaete into this extreme biotope prompted consideration of the metabolic adaptation of this species with regard to its metabolic rate in the presence of H_2S. Therefore we investigated the metabolic rate of different developmental stages of *M. viridis* by means of simultaneous direct and indirect calorimetry.

2. Materials and methods

2.1. Animals

Animals (adults and juveniles) were collected in the Darss-Zingster Bodden chain during 1994 using a sediment corer and a 0.5 mm mesh sieve. Larvae were collected with a planctonic net (120 μm mesh size) in the pelagial. All animals were kept in aquaria containing natural sediment and biotope water with a salinity of 5 ppt with a 12 h light 12 h dark photoperiod. Animals were adapted to the experimental temperature for at least 1 week.

2.2. Metabolic activity

Metabolic activity was determined simultaneously by a Thermometric 2277 microcalorimeter (thermal activity monitoring, Jarfälla, Sweden) and by oxygen sensors (Orbisphere, Geneva, Switzerland) placed at the outflow. Due to the high reaction of sulphide with metals, the measurements were performed using a 20 ml or 4 ml glass perfusion chamber. The flow rate through the system used for adults was 28 ml h^{-1} in the 20 ml system and 8–12 ml h^{-1} in 4 ml perfusion chambers for larvae. The recorded signal was the difference between the heat flow rate of the animal chamber and a reference chamber, which was treated in the same way. Both chambers contained an artificial glass sediment (0.1 mm diameter) (Braun-Melsungen, Melsungen, Germany). Filtered seawater (0.45 μm) was pumped from a reservoir, where oxygen and hydrogen sulphide content were adjusted by a peristaltic pump (IPN4 Ismatec, Glattbruegg-Zürich, Switzerland) placed at the end of the measuring system. Different pO_2 and pH_2S values were obtained

by different sulphide inert gas mixing pumps (Wösthoff, Type: KA18/3a, SA27/3a and 2M300/a, Bochum, Germany). H_2S was a preformed mix of 99% nitrogen and 1% H_2S (Aga-Gas, Berlin, Germany), nitrogen was taken from Messer Griesheim (Rostock, Germany). Single individuals were placed in the chamber. After recording the heat dissipation for 24 h, different settings of experimental conditions followed for periods lasting at least 12 h. After removal of the animals the heat dissipation was measured again to determine the bacterial activity in order to correct the baseline. For correction, heat dissipation and oxygen consumption were recorded without animals under the same conditions.

Heat dissipation and oxygen consumption were recorded by an A/D converter and analysed by a computer with a recording and analysing programme of Baumbach (Berlin, Germany). Experiments were carried out at 10 and 20°C reflecting average winter and summer temperatures.

2.3. Hydrogen sulphide in the sediment

H_2S in the sediment porewater of the habitat was determined during summer and autumn 1994, samples were collected using a glass syringe (1 ml). Samples of 200 μl were fixed in 0.4 ml zinc acetate (0.12 M) and 0.1 ml NaOH (1.5 M) and kept cold until spectrophotometric determination [9,10]. Anoxic solutions were determined by iodometric titration [11].

3. Results

3.1. Hydrogen sulphide in the sediment

Hydrogen sulphide was determined in the porewater of the sediment at two different stations in the Darss-Zingster Bodden chain (Fig. 1). Light symbols represent a location with higher salinity of 6.7 ppt and higher organic load than the location side marked by dark symbols (3.2 ppt). Both stations show a typical depth profile with a steep increase in H_2S in the first centimeters which is maintained or lowered with increasing depth depending on the availability of sulphate or organic material of the sediment. The highest amount of H_2S was found in August 1994 with 3.5 μmol l^{-1} in the first 10 cm of the sediment due to the highest temperature and therefore highest bacterial activity during the summer. The second location marked by dark symbols was generally characterized by a lower sulphide concentration in the sediment due to its lower organic load and salinity.

3.2. Direct calorimetry

A typical time course of the heat flow rate of *M. viridis* (71 mg wet weight) is shown in Fig. 2 at 10°C and 5 ppt. During the first 24 h after inserting the animal in the chamber, peaks of heat dissipation appear occasionally reflecting enhanced locomotory activity whereas the smaller peaks are due to ventilation by the animals. By adding hydrogen sulphide to a final concentration of 520 μmol l^{-1} to the water reservoir, the animals show

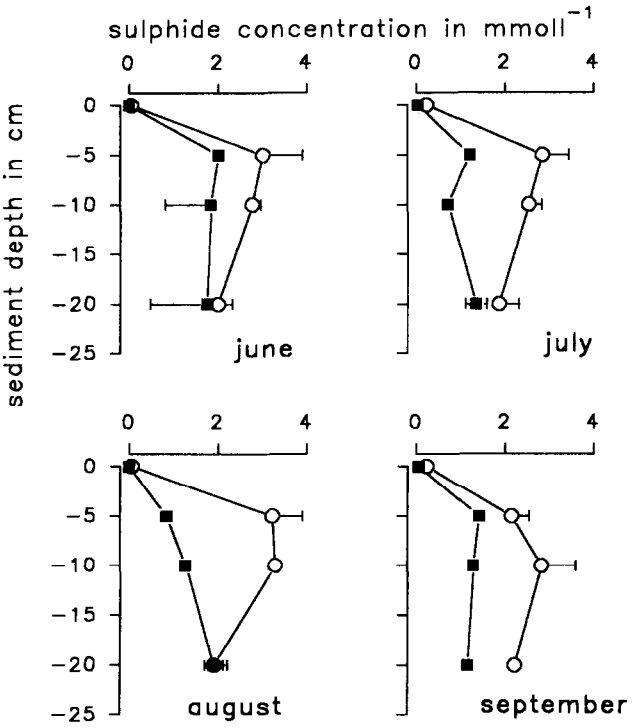

Fig. 1. Amounts of hydrogen sulphide in the sediment porewater depending on the sediment depth at two different places at the Darss-Zingster Bodden chain during summer 1994 indicated by light and dark symbols. The light symbol represents a place with an average higher salinity and higher organic load than the place presented by the dark symbols.

an increased heat flux at moderate hypoxia (14.3 kPa). After 24 h sulphide exposure the animal was removed and the baseline was recorded.

In Fig. 3 part of a time course of *M. viridis* (41 mg wet weight) is shown. After a 36 h period of normoxic incubation, oxygen content was decreased to nominal anoxia. The heat dissipation was depressed immediately to 25% (3.5 μW) of the normoxic heat flux showing fewer and only low activity peaks. Ten hours later H_2S was added to a final concentration of 2.5 mmol l^{-1}. The heat dissipation was slightly raised and a typical enhanced ventilation pattern occurred magnified in the lower part of Fig. 3. After 24 h; H_2S was removed. The reoxygenation caused a sudden rise in heat flow rate which equalled the normoxic heat flow within a period of 6 h.

At moderate hypoxia (8.4–14.3 kPa) the heat flow rate of *M. viridis* was significantly enhanced in the presence of hydrogen sulphide when compared to the control (Fig. 4). The control heat production reached 0.5 mJ mg^{-1} wet wt h^{-1}; with increasing sulphide concentrations the heat flux was raised to 0.7 mJ mg^{-1} wet wt h^{-1} (65 μmol l^{-1}) and 1.015 mJ mg^{-1} wet wt h^{-1} at 225 μmol l^{-1} sulphide. Comparing the directly determined

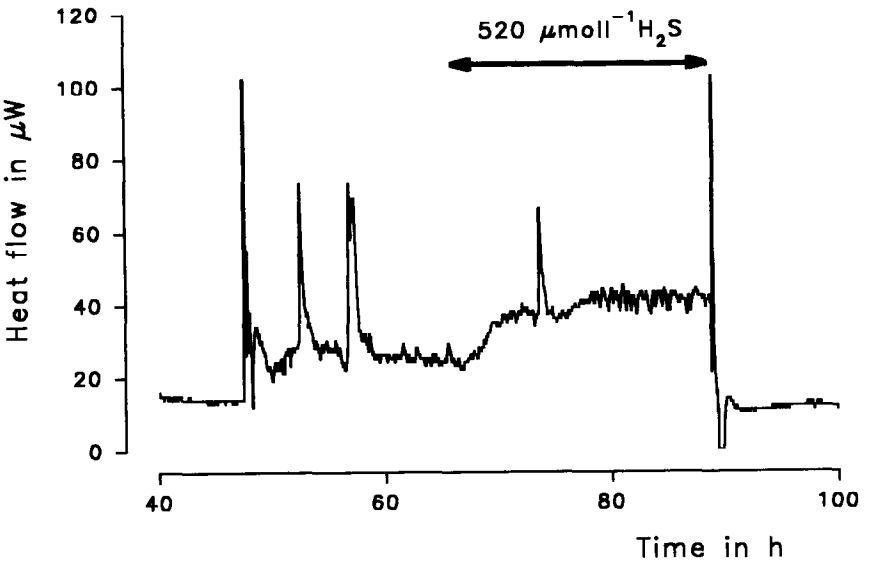

Fig. 2. A typical time course of the heat flow of *M. viridis* with a wet weight of 71 mg at 10°C and a salinity of 5 ppt.

heat dissipation with the oxygen consumption rate of the animals, assuming a caloric equivalent of 450 kJ [12] per mole oxygen which is within the range of the CR ratio determined by [13], the metabolic rate of the animals was fully matched by aerobic metabolism in the presence of hydrogen sulphide.

During autumn, hydrogen sulphide occurs in the water column of the bodden chain and amounts to 160–200 μmol l^{-1} at day time when larvae are obtained. This annual period is characterized by high oxygen and high sulphide input. Surprisingly the relative heat dissipation of larvae at different stages at 10°C and 5 ppt reveal that even larvae of the first 3–6 segments raise their heat dissipation in the presence of low sulphide (0–70 μmol l^{-1}). It is diminished by about 20% when exposed to higher sulphide concentrations (Fig. 5). Larvae with 8–14 segments like the elder larvae increase their heat flow rate to 150% at low sulphide concentration with a constant heat flux up to 250 μmol l^{-1} sulphide.

4. Discussion

The availability of oxygen in coastal sediments can vary considerably, since the oxygen diffusion coefficient in water is small and the increased diffusion distance in interstitial water as well as the high biological and chemical demand of oxygen contribute to keep the pO_2 low. The sediments typically consist of two layers containing a brightly coloured surface stratum followed by a dark, sulfidic smelling sediment [14]. Hydrogen sulphide is generated biotically by microbial sulphate reduction or by microbial degrada-

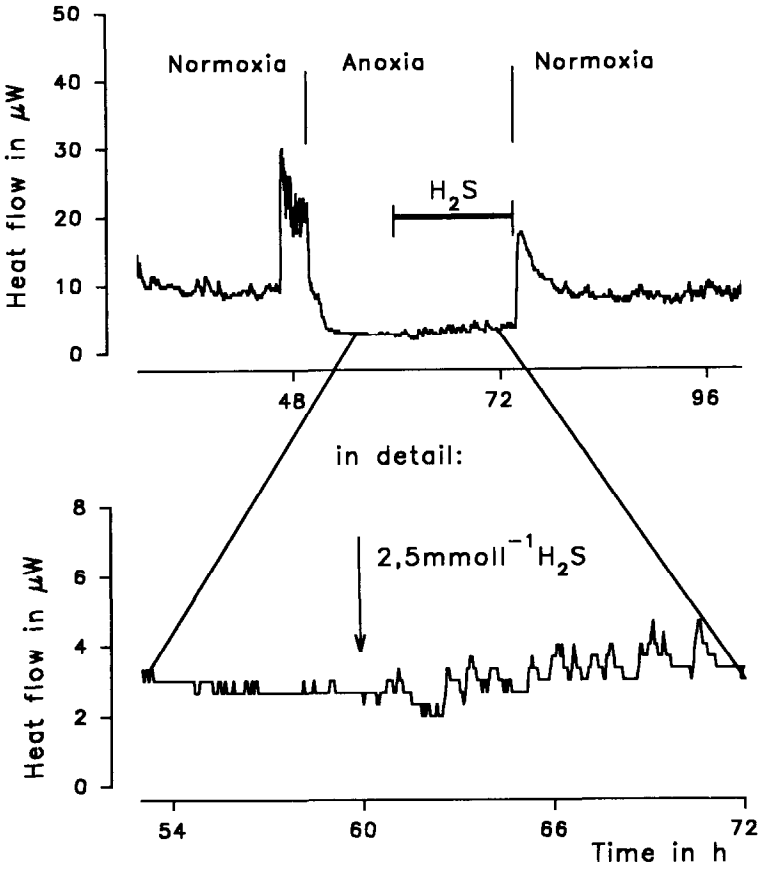

Fig. 3. Part of a time course of the heat flow of *M. viridis* at 10°C and a salinity of 5 ppt and 41 mg wet weight. Heat flow was determined at different pO_2 values; anoxic heat flow is enlarged in the lower part of the figure.

tion of organic material [15]. Corresponding to the salinity and to the high organic load of the Darss-Zingster Bodden chain [5] the observed hydrogen sulphide content (Fig. 1) is within the range of tidal organic loaded marsh sediments in North America and in the Baltic [16–18]. Most burrowing invertebrates in these biotopes are temporarily exposed to hypoxic and sulfidic conditions when the supply of oxygen is interrupted during the night or periods in the summer. The toxicity of hydrogen sulphide is mainly due to its immediate binding to cytochrome c oxidase resulting in a breakdown of aerobic respiration and loss of energy. Secondly, H_2S causes an impairment of metabolites due to changes in enzymes, metabolites and cofactors [19]. Since hydrogen sulphide causes a shut-down of aerobic respiration the metabolic rate was thought to be diminished by increasing hydrogen sulphide. *M. viridis* showed a raised metabolic rate at sulphide concentrations up to 250 μmol l^{-1} which is about 145% of the normoxic control value (Fig. 4) at medium summer temperature (20°C). The metabolic rate was matched fully by aerobic

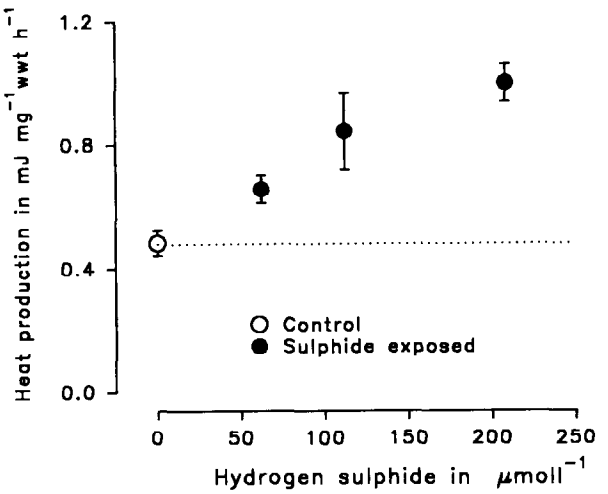

Fig. 4. Heat production of *M. viridis* depending on different sulphide concentrations carried out at a salinity of 5 ppt and 20°C ($n = 3$).

metabolism which was enhanced compared to the controls. One possible explanation for the raised metabolic rate could be the mechanism of detoxification or oxidation of sulphide to less toxic components like thiosulphate, sulphite or sulphate. Sulphide is a highly reduced energy-rich molecule, a variety of biological systems have been evolved to oxidize sulphide to harness its energy [19]. Photoautotrophic and chemoautotrophic bacteria, for instance, use sulphide to gain their energy for carbon fixation or photosynthesis [20,21]. Sulphide-oxidizing bacteria also generate the nutritional energy for many metazoans in sulphidic habitats as described for the plume worm *Riftia pachyptila.* [16]. In addition mitochondria of the intertidal invertebrate polychaetes *Arenicola marina* and *Heteromastus filiformis* [22,23] and the killifish *Fundulus parvipinnes* [24] are capable of sulphide oxidation and generation of ATP. The metabolic rate of *M. viridis* was enhanced in the presence of oxygen and sulphide similar to that described for the mussel *Solemya velum* which harbour symbiotic sulphide-oxidizing bacteria [25].

All these processes are connected by the fact that oxygen has to be available since oxidation of sulphide is only possible if oxygen supply is sufficient. If hydrogen sulphide is oxidized and the additional energy gain is dependent on oxidation, then the anoxic metabolic rate should be maintained. Therefore the metabolic rate of *M. viridis* was determined in the absence of oxygen. Surprisingly the animal showed an elevated ventilation pattern and enhanced its metabolic rate when exposed to 2.5 mmol l^{-1} H_2S (Fig. 3). The slightly elevated anoxic heat dissipation in the presence of high sulphide impact may suggest that the animal can utilize sulphide without oxygen, maybe due to oxidation to sulphur (S^o) which has a lower energy gain than oxidation to S_nO_n components [21]. The mechanism of the additional energy gain could be the involvement of the electron transport chain in the mitochondria which generate ATP or an energy gaining system driven by bacteria. Probably *M. viridis* is capable of sulphide-oxidizing and gaining additional

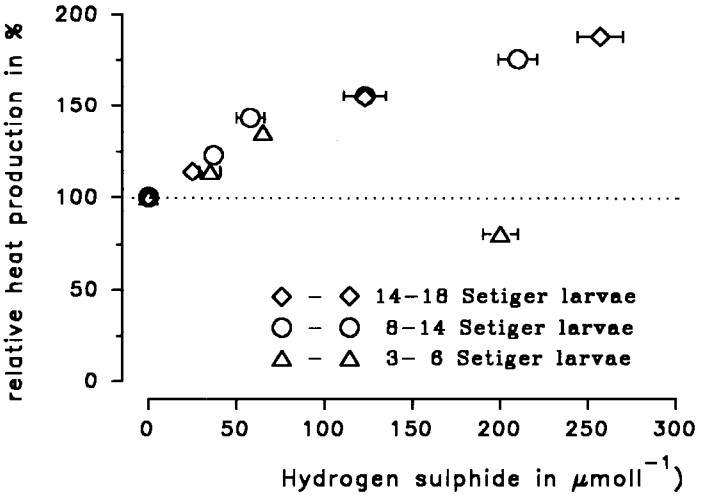

Fig. 5. Relative heat production of different stags of larvae of *M. viridis* depending on sulphide concentration.

energy. If symbiotic or associated bacteria or the animal mitochondria are involved in this process, it needs further research.

The development of the animals account also for the spread and the successful propagation into brackish water habitats. Generally larvae of the early stages are thought to be unable to rely on anaerobic energy processes. If the aerobic respiration is inhibited by sulphide one would expect that the metabolic rate is diminished. Larvae of the blue mussel *Mytilus edulis* and of *M. viridis* have been shown to maintain their metabolism at reduced oxygen levels [26,27]. The metabolic rate of larvae of *M. viridis* was enhanced at low micromolar sulphide concentrations like that of the adults reflecting a strategy which already takes place in the 3-setiger larvae. The resistance of other polychaete and mollusc larvae against low nanomolar sulphide concentration was rather low compared to *M. viridis*, due to the toxic effects of H_2S like inhibition of aerobic respiration [28]. The ability of the larvae of *M. viridis* to survive low micromolar sulphide concentrations occurring in the water column during the pelagic phase reveals that the animal is quite adapted to sulphidic habitats. The resistance of the early stages may be a prerequisite for its rapid propagation into the habitat of the Darss-Zingster Bodden chain.

The metabolic strategies of this polychaete regarding other different abiotic factors such as oxygen availability, salinity and temperature have recently been reported [13,27]. In contrast to the indigenous species *Hediste diversicolor*, *M. viridis* possess a different metabolic strategy for surviving, e.g. hypoosmotic condition at low oxygen and summer temperature.

5. Conclusions

All the developmental stages of *M. viridis* investigated were able to survive hydrogen

sulphide exposure up to 250 μmol l^{-1} when oxygen supply was sufficient. The increased heat dissipation of *M. viridis* in the presence of sulphide may be due to oxidation processes that are possibly connected with an additional energy gain. Aerobic respiration is not inhibited by H_2S concentrations of 520 μmol l^{-1}. Heat flow is slightly enhanced under anoxic conditions suggesting an energy gaining process which may be different to that at hypoxia or normoxia. The advantage of this adaptation to sulphide may be that the animal, and already its developmental stages, are capable of surviving in sulphidic environments and spread into habitats which are inhabited by only a few species. This may be an explanation for its rapid propagation in Baltic shallow coastal waters where hydrogen sulphide frequently occurs in the water column and is commonly present in the sediment.

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