Rates of sediment sulphide oxidation by the bivalve mollusc *Thyasira sarsi*

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ABSTRACT: Several bivalve molluscs, including species of *Thyasira* (family Thyasiridae), use symbiotic sulphur-oxidising bacteria to exploit sulphides in reducing sediments are able to oxidise the insoluble sulphides as well as dissolved sulphide. The behaviour of these bivalves was observed in narrow glass vessels. Rates of sulphide oxidation by *Thyasira sarsi* (Philippi) were determined in a mesocosm, using specimens and sediment from Oslofjord. The rate of oxidation of reduced sulphur attributable to *T. sarsi*, 8.7 mmol d^{-1} m^{-2}, was close to the rate of sulphide formation in the same sediment, 8.2 mmol d^{-1} m^{-2}. This suggests that sulphate reduction rates control density in *T. sarsi* populations. The activities of *T. sarsi* and other lucinaceans can re-oxidise reducing and polluted sediments, allowing colonisation by sulphide-intolerant benthic animals.

KEY WORDS: *Thyasira sarsi* · Sulphide oxidation · Sulphate reduction · Bioturbation · Population density · Ecosystem engineering

INTRODUCTION

Bacterial sulphate reduction is a major pathway for the mineralization of organic matter in marine sediments (Jørgensen 1989). The sulphide accumulates as iron sulphides and, when available iron has become depleted, free sulphide. Bivalves of the genus *Thyasira* use their extremely extensible foot to construct deep burrows beneath their shell (Allen 1958, Dando & Southward 1986) to take up free sulphide from deep in the sediment. The sulphide is utilised by chemoautotrophic, sulphur-oxidising bacteria, housed in the gills of several thyasirids, including *Thyasira sarsi* (Philippi), and similar symbiotic bacteria occur in other deep-burrowing lucinaceans (Dando & Southward 1986, Southward 1986). Several other lucinaceans form deep burrows in the sediment below the shell (Stanley 1970). Recently reported experiments (Dufour & Felbeck 2003) confirm the burrowing behaviour of *Thyasira* and show that the burrows are deeper where free sulphide levels are low.

Thyasira sarsi reaches a maximum size of 25 mm and is found in organic-rich shelf sediments along the Nordic coasts from the Kattegat to the Lofotens (Ockelmann 1961). It can reach densities of several thousand m^{-2} in sewage-enriched sediments (Dando & Southward 1986) and, with *T. flexuosa*, is an indicator of organic enrichment (Pearson & Rosenberg 1978). *T. sarsi* was formerly rare in the open North Sea, being reported only from natural methane seeps (Dando et al. 1991, 1994a), but has recently colonised oil-containing sediments close to the well heads of North Sea oil rigs (Oliver & Killeen 2002).

Both *Thyasira sarsi* and *T. flexuosa* burrow into soft sediments to a maximum depth of approximately 9× their shell length, giving a burrowing depth of 180 mm for a 20 mm long individual (Dando & Southward 1986). The thyasirids lack a siphon and use the foot to construct an anterior inhalant canal by cementing sediment particles together with mucus (Allen 1958). Oxygenated water is drawn through this canal, for respiration and heterotrophic feeding, and is then discharged into the sediment. *T. sarsi* commonly obtains most of its carbon from the symbiotic bacteria (Dando & Spiro 1993), but it can occur in many habitats where there is no detectable free sulphide (Dando & Southward 1986). We have carried out experiments to demonstrate how *T. sarsi* can obtain sulphide for its

symbiotic bacteria in sediments where free sulphide is lacking and to measure the rate at which this bivalve could oxidise insoluble sulphur species in the sediment.

MATERIALS AND METHODS

Thyasira sarsi collected from the inner Grimstadfjord near Bergen, Norway, were allowed to burrow in 20 cm tall glass-fronted chambers of 1 cm width, in which the faceplates were covered by black acrylic slides that could be removed to permit observation. These chambers were filled with sediment from the habitat. Specimens of *T. sarsi* were placed on top of the sediment and the chambers immersed in a deep tank of circulating seawater at the Marine Biological Association (MBA) laboratory, Plymouth. The observation chambers were removed from the seawater after 5 d and again after 15 d, the slides lifted and the tunnel structures photographed.

For the study on chemical transformations effected by the bivalves and their symbionts, box cores of sediment were collected on 23 September 1986, using a U.S. Naval Electronics Laboratory design (USNEL) spade-box corer, operated from RV 'Trygve Braarud'. The box cores had an area of 0.222 m^2, and a depth of 50 cm, and were taken from 39 m deep in a known habitat of *Thyasira sarsi* in Vestfjorden, Oslofjord, Norway (59° 47.01' N, 10°30.87' E). The station was in a slight depression with a 15 m high rock sill to the south. All the box cores were taken in the same position on the same day. The retrieved cores, in PVC liners, were placed in flowing seawater in the mesocosm facility at the Norsk Institut for Vannforskning (NIVA), Solbergstrand. Seawater, at 11.0 ± 0.9°C, was pumped into the mesocosm tank from 45 m depth in Oslofjord, and there was 1 m of water above the top of the box cores. Daylight was excluded. PVC tubes (150 mm long, 103 mm internal diameter) were pressed into each experimental box core to form subcores. The top of the tube was temporarily fitted with a transparent stopper through which a slight vacuum was applied as the tube was very slowly pressed into the sediment until only a few mm protruded (Fig. 1b,c).
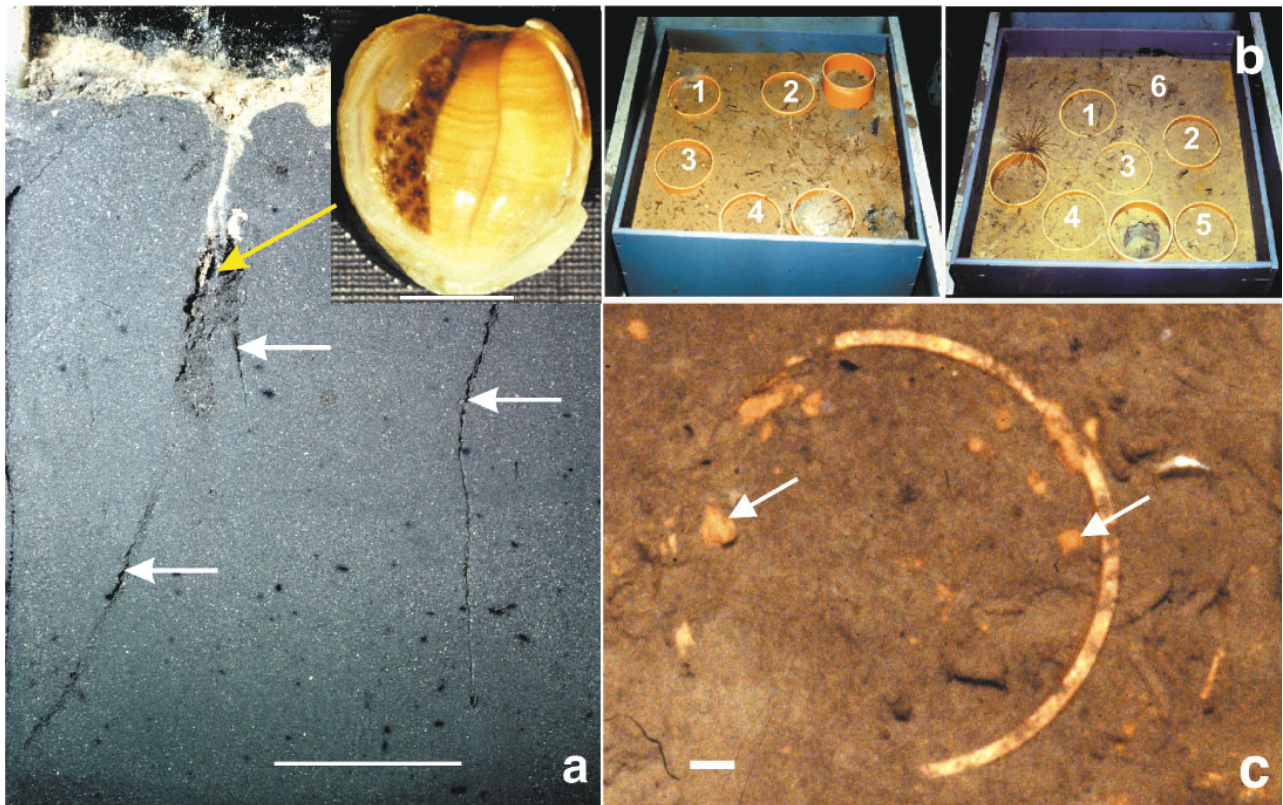

Fig. 1. Tanks and cores containing *Thyasira sarsi*. (a) Tunnel systems formed in the sediment by *T. sarsi* after 15 d: tunnels below the bivalve (white arrows); oxidation of the sediment reveals the position of the vertical inhalant tube (yellow arrow). Inset is a specimen with the left valve removed, showing the sulphur-laden gills (scale bar = 10 mm). (b) Box Cores 1 (left) and 2 (right) during the experiment, showing numbered subcores (subcores that are not numbered were not used in this study). A glass bottle was found in the centre of Box Core 1 when it was recovered and this part of the core was not used. (c) Close-up of one insert showing the mounds of hydrated iron (arrowed) over the exhalent streams from the bivalves (scale bar = 10 mm)

This slight vacuum prevented compression of the sediment and minimised disturbance during tube insertion. The sediment was not otherwise disturbed and no attempt was made to kill or remove existing fauna. However, the sediment disturbance resulting from placing the box cores in the mesocosm and inserting the tube sections caused some thyasirids to come to the surface during the 2 d following the disturbance. These bivalves subsequently burrowed into the sediment again. Sediment from one of the box cores was sieved to obtain live *T. sarsi*; 8 of the freshly removed individuals were immediately dissected for measurement of elemental sulphur (Fliermans & Brock 1973). A total of 90 live bivalves with no visible shell damage were sorted into 3 groups of 10 and 3 groups of 20, with assorted sizes in each group. These groups were added to randomly chosen sub-cores (Table 1) in an attempt to change the densities of the bivalve in the core sections to values equivalent to approximately 1000 or 2000 *T. sarsi* m^{-2}. The final number of individuals present could not be determined until the end of the experiment, since some of the added thyasirids may have died after burrowing, others may have moved over the shallow core lip before burrowing and there was an unknown number in each core section originally. We found that there had been a high mortality in, or migration from, the densely stocked subcores (Table 1).

After 30 d the tubes were removed and the subcores sectioned into 15, 20 or 30 mm slices. The sediment sections were pushed out of the subcore tubes into argon-filled bags, which were kneaded to homogenise the sediment before chemical sampling. Pore water was obtained by pressure filtration through cellulose acetate filters of 0.2 μm porosity. Bivalves were removed from the sediment by washing on a sieve of 1 mm^2 mesh. Dissolved sulphide was determined by

colorimetry (Cline 1989) and sulphate by HPLC. Sediment sub-samples were extracted with hexane and the evaporated extracts were analysed for elemental sulphur (Fliermans & Brock 1973). Acid-labile sulphide and chromous-reducible sulphide were then determined by reducing the sulphur to hydrogen sulphide in stages (Zhabina & Volkov 1978) and measuring the sulphide by colorimetry (Cline 1989). Sulphate reduction rates were determined by injection of ^{35}S-sulphate into 3 ml subcores, which were incubated at 10°C for 19 h (Dando et al. 1991). pH was measured to ±0.1 pH unit with a combination electrode calibrated with National Bureau of Standards (NBS) buffers. Sampling + analytical precision was ±2% for dissolved sulphide, ±1% for sulphate, ±5% for insoluble sulphur species and ±10% for sulphate reduction.

RESULTS

The nature of sediment modification by *Thyasira sarsi* was shown by the specimens placed in the narrow chambers containing reduced sediment from their habitat (Fig. 1a). Within 5 d, the sediment surrounding the inhalant canals was paler and tunnels were obvious in the sediment deep below the position of the shell. After 15 d the sediment around the inhalant canals was yellow, indicating that the particulate iron sulphides in the surrounding sediment had been oxidized.

Quantification of the effect of the bivalves on the sediment was provided by the experiments in Norway, where freshly collected specimens of *Thyasira sarsi* were added to subsections of box cores (see 'Materials and methods'). The interstitial water in the upper 50 cm of sediment contained less than 0.1 μM total dissolved sulphide; 90% of the *T. sarsi* were in the upper 50 mm (Table 2). Before additional specimens were added, the box core sediment contained *T. sarsi* at a density equivalent to 411 m^{-2} and specimens were in the size range of 2.7 to 8.5 mm shell length, mean

Table 1. *Thyasira sarsi.* Number of individuals added to each subcore in the mesocosm experiment. The original number of *T. sarsi* in each subcore was unknown. The number recovered by sieving the sediment at the end of the 30 d study is shown in the final column

Box core	Subcore	No. added	No. after 30 d
1	1	10	14
1	2	20	11
1	3	0	5
1	4	0	2
2	1	10	16
2	2	20	15
2	3	20	16
2	4	10	17
2	5	0	1
2	6	0	4

Table 2. *Thyasira sarsi.* Number of individuals found at different sediment depths in the subcores

Sediment depth (cm)	Box Core 1	Box Core 2
0–1.5	7	21
1.5–3.0	16	26
3.0–5.0	6	14
5.0–7.0	2	5
7.0–9.0	0	0
9.0–12.0	1	0
12.0–14.5	0	1

5.2 ± 1.8 (SD) mm. The 10 experimental subcores were divided into 2 groups for data analysis; 4 subcores formed the 'low-density' group with 1 to 5 *T. sarsi* per core (mean: 3.3 *T. sarsi* per subcore, equivalent to 333 m^{-2}) and 6 subcores formed the 'high-density' group with 11 to 17 *T. sarsi* per core (mean: 14.7, equivalent to 1630 m^{-2}).

The pH of the expressed interstitial water (Fig. 2a) was lower in the 'high-density' group at all 7 sediment depth levels examined (sign test, $p < 0.01$). The greatest decrease, from a mean of pH 7.39 to a mean of pH 6.52, was in the top 15 mm layer. Acid-labile sulphide (mainly FeS) and chromous-reducible sulphide (FeS$_2$) concentrations were less at all depths in the 'high-density' subcores compared with the 'low-density' subcores ($p < 0.01$). With the exception of the 5 to 7 cm depth section the elemental sulphur concentrations were always lower in the 'high-density' group ($p < 0.016$). At the bottom of the subcores, below 12 cm depth, the concentrations of reduced sulphur species converged in the 2 groups (Fig. 2b). A cumulative plot of the total insoluble reduced sulphur (Fig. 2c) showed that the 'high-density' subcores had a mean reduced sulphur content, to 14 cm depth in the core, of 44.0 mmol subcore^{-1} compared with 51.4 mmol subcore^{-1} for the 'low-density' subcores. This difference, of 7.4 mmol core^{-1}, in subcores with an average of 11.7 more *Thyasira sarsi*, equals loss from the sediment, of 21.3 μmol reduced sulphur d^{-1} for every *T. sarsi*. This is equivalent to a loss of 8.7 mmol of reduced S d^{-1} m^{-2} at the density of 411 *T. sarsi* m^{-2} at the study site in Oslofjord.

Sulphate reduction rates were determined over the upper 14 cm of sediment of the 'low-density' Subcore 2-6, containing 4 *Thyasira sarsi*. The integrated rate was equivalent to 8.2 mmol sulphate reduced d^{-1} m^{-2}. The rate was fairly uniform throughout the subcore, with the exception of the partially oxidised upper 15 mm (Fig. 3). Reduction rates were determined on 2 sections of a 'high-density' core (2-1) containing 16 *T. sarsi*. The similarity of rates at 5 to 9 cm sediment depth (Fig. 3), 0.067 mmol sulphate reduced dm^{-3} sediment d^{-1} for the 'low-density' core and 0.065 to 0.073 mmol sulphate reduced dm^{-3} sediment d^{-1} for the 'high-density' core, suggests that increasing the number of bivalves did not substantially alter the rate of sulphide formation.

The gills contained up to 37.8 ± 14.4 (SD) μmol elemental sulphur g^{-1} wet wt of gill tissue, with the highest concentrations being found in the largest bivalves and in bivalves with the highest ratios of (gill weight):(total tissue body weight) (Fig. 4). Elemental sulphur was not found in non-gill tissues, nor was it found in gill or non-gill tissues of other bivalves in this sediment (data not shown).
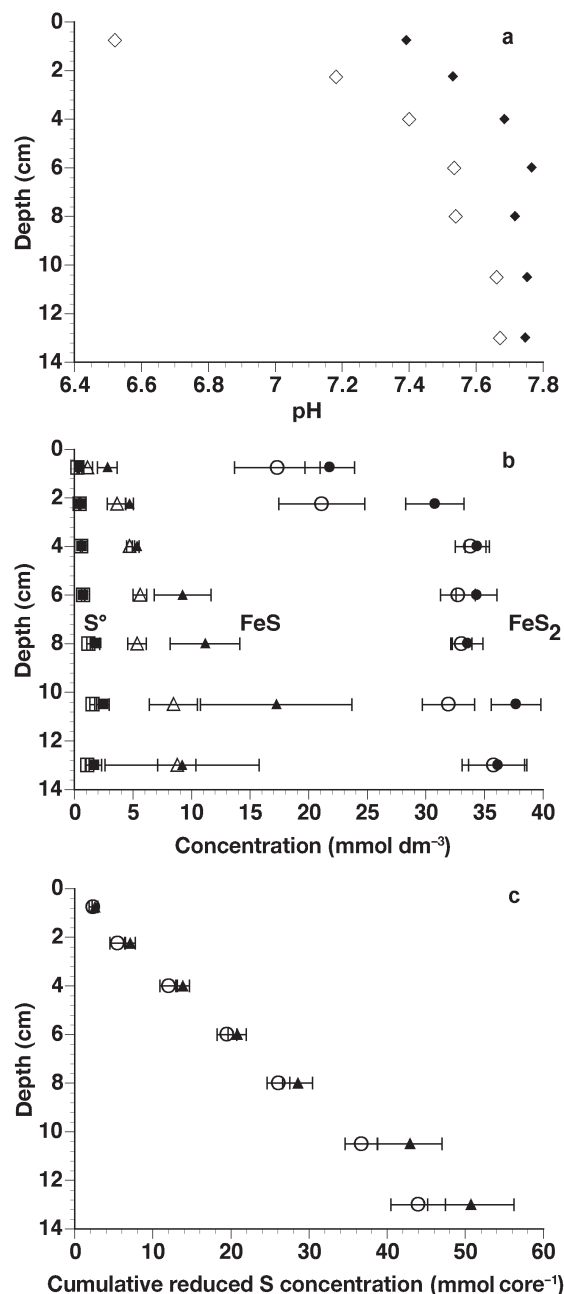

Fig. 2. Sediment chemistry profiles for subcores with *Thyasira sarsi.* Solid symbols: 'low' numbers (1 to 5 ind.); open symbols 'high' numbers (11 to 17 ind.). (a) Mean pH. (b) Concentrations of insoluble reduced sulphur, elemental sulphur (S^0) (squares), acid labile sulphide (FeS) (triangles), and chromous reducible sulphide (FeS$_2$) (circles); all data are mean ± SE. (c) Cumulative concentration, with depth, of reduced sulphur in the core tubes (S^0 + FeS + FeS$_2$); mean ± SE

DISCUSSION

Thyasira sarsi normally obtain much of their nutrition from the endosymbiotic sulphur-oxidising bacteria in their gills. The gills are cream-coloured from de-
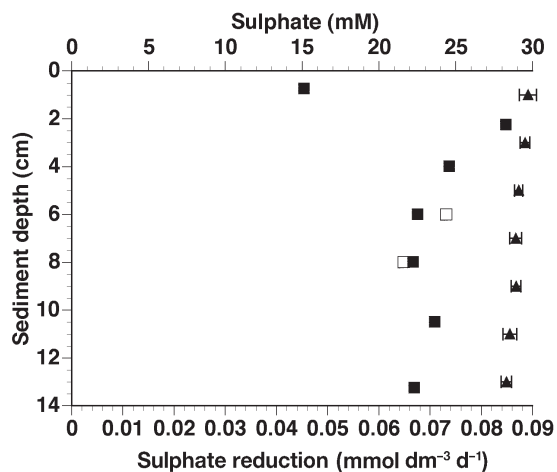
Fig. 3. Sediment sulphate reduction rates (squares) and interstitial water sulphate concentrations (triangles) in Subcores 2-6 (closed symbols) and 2-1 (open symbols)

position of elemental sulphur in invaginations of the bacterial membranes. *T. sarsi* from methane seep habitats and from very organic-rich fjord sediments, where free sulphide is readily available, have tissues that are highly depleted in ^{13}C, showing δ^{13}C values of -31 to $-39.5‰$ (Spiro et al. 1986, Schmaljohann et al. 1990, Dando et al. 1991, Dando & Spiro 1993), indicating that most of their nutrition comes from the endosymbiotic bacteria. The most depleted value found for non-methane seep sediments was $-34‰$ δ^{13}C. Lower δ^{13}C values in animals collected around methane seeps probably resulted from some of the inorganic carbon fixed being derived from highly ^{13}C-depleted methane. Detailed electron microscopy studies (Schmaljohann 1991) have provided no evidence that *T. sarsi* close to methane seeps has a dual

symbiosis of methanotrophic symbionts as well as sulphur-oxidising symbionts, unlike the situation in some species of *Bathymodiolus* (Fiala-Médioni et al. 2002).

In sediments in which there was no odour of hydrogen sulphide and where it could not be detected by colorimetry (i.e. concentration <0.1 µM; Dando et al. 1985), the ^{13}C content of *Thyasira sarsi* tissues was much higher. In some specimens it approached the values found in heterotrophic bivalves in the same sediment, -17.4 to $-17.1‰$ δ^{13}C (Dando & Spiro 1993). This implies a lesser dependence on organic carbon supplied by the endosymbionts to *T. sarsi* in such sediments. The *T. sarsi* from the Oslofjord sediment used in this study had mean tissue δ^{13}C values of -22.4 to $-24.0‰$ (Dando & Spiro 1993). If we assume a δ^{13}C of $-18‰$ for heterotrophic bivalves in the fjord and a value of $-34‰$ for a *T. sarsi* entirely dependent on endosymbionts for nutrition, then these Oslofjord bivalves are obtaining between 27.5 and 37.5% of their tissue carbon from the bacteria. The gills of all but one of the *T. sarsi* specimens examined contained elemental sulphur, confirming that reduced sulphur species were being taken up. The increasing elemental sulphur content with increasing shell size (Fig. 4) was probably related to the deeper burrowing capability of the larger individuals and hence their access to greater amounts of reduced sulphur.

The above evidence indicates that the sulphur-oxidising endosymbiotic bacteria in this Oslofjord population of *Thyasira sarsi* contributed significantly to their nutrition. A response of *T. sarsi* to low free sulphide in the sediment is to extend the length and depth of the burrow below the shell to access higher concentrations of sulphide (Dufour & Felbeck 2003). However, such burrows are limited to a maximum depth of 30× the shell length, which would be 255 mm in the pre-
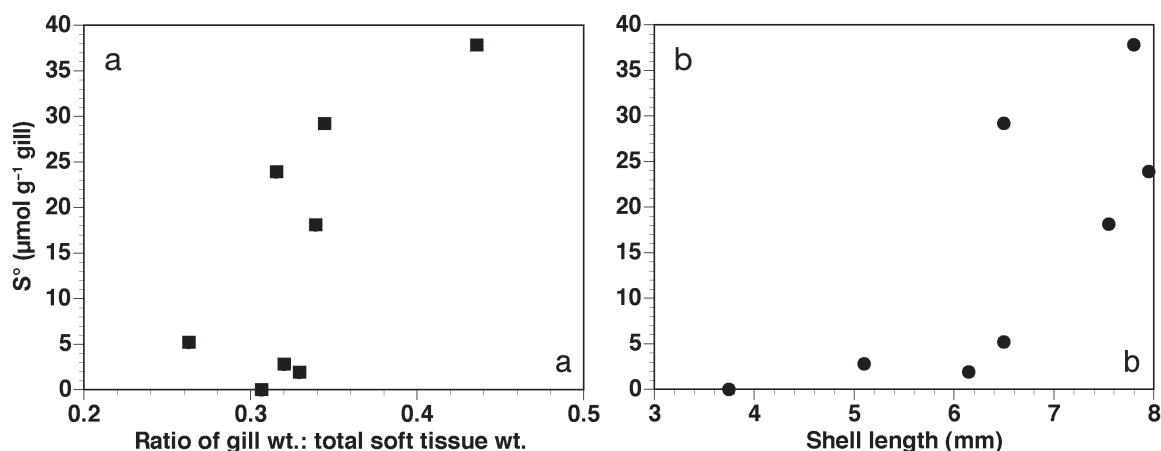

Fig. 4. *Thyasira sarsi.* Elemental sulphur content of the gills, showing the variation with (a) relative gill weight, (b) shell length

sent study, and free sulphide was still undetectable at 500 mm depth in our sediment samples. This suggested that, as described for *Lucinoma borealis* (Dando et al. 1994b), *T. sarsi* are able to 'mine' the insoluble sulphur species in the sediment by drawing oxygenated water through a semi-permeable inhalant tube. The results of the mesocosm experiment demonstrated that adding *T. sarsi* to otherwise undisturbed sediment lowered the concentrations of acid-labile sulphide, chromous-reducible sulphide and elemental sulphur in the sediment above the bivalves. The overall reduction in insoluble reduced sulphur resulting from activity of *T. sarsi* in the sediment was equivalent to 8.7 mmol of reduced S d^{-1} m^{-2}.

The rate of sulphide formation in the mesocosm experiment was 8.2 mmol d^{-1} m^{-2}. Although sulphate reduction determinations were only made on 2 subcores, the rate was identical, within measurement error, to the rate of removal of reduced sulphur by *Thyasira sarsi*. We suggest that this similarity is not coincidental, but that the sulphate reduction rate of sediment determines the maximum sustainable density of symbiont-containing thyasirids.

At the 39 m site in Vestfjorden the bottom water does not become deoxygenated, defaunation does not occur and thyasirid densities are relatively stable (Aschan & Skullerud 1990). In contrast, at fjord sites where the bottom water can become deoxygenated there are large variations in thyasirid densities (Dando & Spiro 1993). At such places, the pulse of dead organic matter added to the sediment following infaunal death, plus the deoxygenation of the bottom water, increases the sulphate reduction rate. When oxygenated water is introduced, a large thyasirid population rapidly becomes established and the rate of sulphide oxidation by this population will exceed the declining sulphate reduction rate. Under these conditions sediment sulphides are rapidly depleted. When sulphide sources are low, some of the thyasirids can exist heterotrophically for a period (Dando & Spiro 1993).

As with other lucinaceans (Dando et al. 1994b), pumping oxygenated water through the semi-permeable inhalant tube results in rapid oxidation of the iron sulphides in the surrounding sediment. Disproportionation of the thiosulphate that is formed under these conditions results in free sulphide becoming available and diffusing back into the tube. The thyasirids also have a posterior inhalant aperture that draws water directly from the sediment onto the gills (Allen 1958). The fluid flows induced in the tunnels (Oliver & Killeen 2002) and by 2 inhalant streams (Allen 1958) will produce a considerable passage of water through the sediment, increasing the oxidation and removal of sulphur species. The very slight depletion of sulphate with depth in cores with few *Thyasira sarsi*, i.e. with

low sulphide oxidation rates despite the high sulphate reduction rate (Fig. 3), provides additional evidence for enhanced fluid flow and re-oxidation of sulphides. Sulphuric acid, excreted by the sulphur-oxidising bacteria in the gills, will be expelled into the sediment in the exhalant water stream, further increasing solubilisation of the sulphides.

Symbiont-containing thyasirids are secondary colonisers of organic-rich sediments, coming after capitellid polychaetes that feed directly on bacteria (Pearson & Rosenberg 1978). The thyasirids are in turn followed by the larger lucinids. The term 'ecosystem engineer' has been applied to those organisms that modulate the availability of resources to other species (Mitsch & Jörgensen 1989, Jones et al. 1994). The activities of the thyasirids reported here would qualify them as ecosystem engineers of the allogenic type (Jones et al. 1994). It has been suggested that large aggregations of symbiont-containing perviate and obturate pogonophorans likewise can modify the sediment chemistry (Southward & Dando 1988, Cordes et al. 2003), albeit on a much slower time scale. The activity of the symbiont-containing bivalves in irrigating sediments removes toxic hydrogen sulphide and creates conditions for the successful colonisation of the sediment by heterotrophic species. This natural response of the ecosystem might well be exploited to regenerate organically polluted sediments. Bioturbation by later burrowers, such as polychaetes, crustaceans and infaunal echinoderms that follow the bivalves, will disrupt the tunnel systems, decreasing the ability of the bivalves to obtain sulphide. This helps to explain why *Thyasira sarsi*, *T. flexuosa* and other lucinaceans are most abundant in sediments with an otherwise sparse infauna (Allen 1958).

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