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Metabolite uptake, stoichiometry and chemoautotrophic function of the hydrothermal vent tubeworm *Riftia pachyptila*: responses to environmental variations in substrate concentrations and temperature

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Summary

The hydrothermal vent tubeworm *Riftia pachyptila* is a dominant member of many hydrothermal vent communities along the East Pacific rise and is one of the fastest growing metazoans known. *Riftia* flourish in diffuse hydrothermal fluid flows, an environment with high spatial and temporal heterogeneity in physical and chemical conditions. To date, physiological and biochemical studies of *Riftia* have focused on *Riftia's* adaptations to its chemoautotrophic bacterial symbionts. However the relation between *in situ* physico-chemical heterogeneity and *Riftia* host and symbiont metabolism, in particular symbiont chemoautotrophic function, remain poorly understood. Accordingly, we conducted experiments using shipboard high-pressure respirometers to ascertain the effect of varying substrate concentrations and temperature on *Riftia* metabolite uptake and symbiont carbon fixation. Our results show that substrate concentrations can strongly govern *Riftia* oxygen and sulfide uptake rates, as well as net carbon uptake (which is a proxy for chemoautotrophic primary production). However, after sufficient exposure to sulfide and oxygen, *Riftia* were capable of sustaining symbiont autotrophic function for several hours in seawater devoid of sulfide or oxygen, enabling the association to support symbiont metabolism through brief periods of substrate deficiency. Overall, temperature had the largest influence on *Riftia* metabolite uptake and symbiont autotrophic metabolism. In sum, while *Riftia* requires sufficient availability of substrates to support symbiont chemoautotrophic function, it is extremely well poised to buffer the temporal and spatial heterogeneity in environmental substrate concentrations, alleviating the influence of environmental heterogeneity on symbiont chemoautotrophic function.

Key words: metabolism, stoichiometry, *Riftia*, hydrothermal vent, chemoautotrophy, symbiosis.

Introduction

Riftia pachyptila (hereafter referred to solely as *Riftia*) is a monospecific genus within the family Siboglinidae (Rouse, 2001) and is indigenous to the vent fields of the Eastern and Southeastern Pacific (Shank et al., 1998). *Riftia* is the dominant megafaunal species at many sites, often growing in enormous aggregations and hosting numerous other species such as mussels, polychaete worms, limpets and crabs (Hessler et al., 1988; Shank et al., 1998; Tunnicliffe, 1991; Govenar et al., 2005). *Riftia* is devoid of a mouth or digestive tract, and possesses intracellular chemoautotrophic bacteria within a vascularized organ called the trophosome (Cavanaugh et al., 1981; Felbeck, 1981). *Riftia* cannot ingest particulate organic matter, and flourishes where dissolved organic carbon and nitrogen concentrations are too low to support the observed biomass (Johnson et al., 1988; Gaill et al., 1997). As such, *Riftia* relies entirely on its symbionts for nutrition. Because the symbionts are not in contact with the external milieu, all their substrates and waste products are provided for or eliminated by the host *Riftia*.

Accordingly, *Riftia* must acquire both reduced and oxidized substrates for chemoautotrophic metabolism and therefore thrives in diffuse flow regimes, positioning its plume-like gill at the interface of vent flow and bottom-water mixing (Childress et al., 1991). However, this niche is spatially and temporally heterogenous (Johnson et al., 1986). Environmental chemistry in diffuse flows is wildly variable on short-time scales (Johnson et al., 1986; Johnson et al., 1988), with dissolved inorganic carbon concentrations ranging from 2 to >12 mmol l^{-1}, hydrogen sulfide from undetectable to 725 μmol l^{-1}, and dissolved oxygen and nitrate concentrations ranging from 0 to 110 μmol l^{-1} and 0 to 40 μmol l^{-1}, respectively (Shank et al., 1998; Luther et al., 2001; Mullineaux et al., 2003; Le Bris et al., 2006). Temperatures at

diffuse flow sites have been observed to vary between 2 to 25°C, and also to change rapidly over time (Chevaldonne et al., 1991; Johnson et al., 1988).

Prior physiological studies of *Riftia* have largely focused on characterizing the physiological and biochemical adaptations of host to symbiont, as well as elucidating which metabolites are used by the symbioses (Arp, 1988; Arp and Childress, 1983; Childress et al., 1984; Childress and Fisher, 1992; Childress et al., 1993; Felbeck et al., 1981; Fisher and Childress, 1984). To date, little is known about how environmental conditions such as metabolite concentrations, pH and temperature influence the metabolism (and ultimately growth) of *Riftia* and its symbionts. The aforementioned spatial and temporal environmental variability makes it impractical to ascertain such relationships *in situ*. Accordingly, the experiments presented here examined the relation between *Riftia* metabolite uptake, symbiont chemoautotrophic function, seawater metabolite concentrations, pH and temperature using a shipboard high-pressure respirometry system. We conducted our experiments over a range of environmentally relevant chemical concentrations and temperatures to examine how thermal and chemical fluctuations *in situ* might influence host metabolite uptake and symbiont autotrophic function. We also examined the stoichiometric relations among the major metabolites, as well as which chemical species are preferentially acquired by *Riftia*.

Materials and methods

Study sites and collection methods

All experiments were conducted on board the *R/V Wecoma* or *R/V New Horizon* during expeditions in April and May 1996 (HOT 96), November and December 1997 (HOT 97) and November and December 1998 (LARVE 98). *Riftia pachyptila* Jones tubeworms were collected from two hydrothermal vent fields along the East Pacific Rise (12°48′N,103°56′W, and 9°50′N,104°18′W), at a depth of about 2300 m. Tubeworms were collected daily by the *DSV Alvin* or *DSV Nautile* and brought to the surface in a thermally insulated container (Mickel and Childress, 1982). After arrival on board ship, the tubeworms most responsive to touch were immediately placed into flow-through, high-pressure respirometer aquaria, where they were maintained in 0.2-μm filter-sterilized flowing seawater for 1–2 h at 10°C and 27.5 MPa (Girguis et al., 2000).

Experimental apparatus

In all respirometry experiments, two of the pressurized aquaria contained tubeworms while the third served as the control. To simulate the seawater chemistry found *in situ*, 0.2-μm filter-sterilized seawater was pumped into an acrylic gas equilibration column and bubbled with carbon dioxide, hydrogen sulfide, oxygen and nitrogen or helium to achieve the desired dissolved gas concentrations (Kochevar et al., 1992). Seawater pH was adjusted by using a proportional pH controller and isoosmotic HCl and NaOH solutions (Prominent Inc., Pittsburgh, PA, USA). A sodium nitrate solution (in 0.2-μm filtered-sterilized seawater) was pumped into the equilibration column to produce final seawater nitrate concentrations between 40 and 65 μmol l^{-1}. Seawater from the equilibration column was delivered to the three aquaria by high-pressure pumps (American Lewa, Inc., Holliston, MA, USA). High-pressure aquaria temperatures were maintained at 15°C by immersing them in a circulating waterbath, while aquaria pressures were maintained at 27.5 MPa *via* diaphragm backpressure valves (Circle Seal, Inc., Corona, CA, USA). Vessel effluents were directed through a computer-controlled stream-selection valve that diverted one stream to the analytical instrumentation every 7 min.

During the HOT 96 and HOT 97 expeditions, the analytical system consisted of a membrane-inlet quadrupole mass spectrometer to determine all dissolved gas concentrations, an inline pH electrode and a spectrophotometer for nitrate analyses (Girguis et al., 2002; Girguis et al., 2000). During the LARVE 98 expedition, inorganic carbon concentrations were measured using a carbon dioxide specific electrode (pHoenix, Inc., Houston, TX, USA) mounted in a water-jacketed flow-through cell. Hydrogen sulfide concentrations were determined by a quantitative spectrophotometric assay (Guenther et al., 2001) using a Gilson spectrophotometer with a 250 μl flow-through cell. Oxygen concentrations were determined by a silver/silver chloride electrode (Cameron Instruments Inc., Guelph, ON, Canada) mounted in a 2 ml flow-through cell. All carbon dioxide, hydrogen sulfide and oxygen measurements were confirmed and calibrated using a Hewlett-Packard 5890 Series II gas chromatograph (Childress et al., 1984). During both experiments, pH was measured using a double-junction pH electrode mounted in a water-jacketed flow-through cell, and connected to Orion model 920A or Radiometer PHM 93 pH meter, while nitrate was analyzed from discrete samples collected every 30 min using a quantitative spectrophotometric assay (Girguis et al., 2000; Karlsson et al., 1995). Temperature was measured and recorded by a digital thermometer (Fisher, Inc., Hampton, NH, USA).

Riftia acclimation and sampling, pre- and post-experimentation

Prior to all experiments, *Riftia* were placed in the respirometer aquaria, and were maintained in conditions typical of those *in situ.* These 'typical' conditions are: total dissolved inorganic carbon (i.e. ΣCO_2)=5.5–6 mmol l^{-1}, total dissolved sulfide (i.e. ΣH_2S)=250–300 μmol l^{-1}, dissolved O_2=90–180 μmol l^{-1}, dissolved NO_3=40–50 μmol l^{-1}, pH=6.5, temperature=12°C, pressure=27.5 MPa. *Riftia* were maintained in these conditions until 'autotrophic'. Autotrophic *Riftia* exhibit a net uptake of dissolved inorganic carbon (DIC), oxygen and sulfide, as well as net elimination of proton equivalents. This regularly occurs after 12 h following incubation.

During each experiment, while one or more factors were being varied, all other dissolved substrate concentrations, as well as pH and temperature, were held at the 'typical' conditions previously described. Also during all experiments, tubeworms were maintained at each interval for at least 1 h, or until uptake rate reached a steady state.

At the end of each experiment, worms were promptly removed, weighed on a motion-compensated shipboard balance (Childress and Mickel, 1980), dissected and frozen in liquid nitrogen for later analysis. In some cases, empty worm tubes were returned to the pressure vessels for several hours, and subjected to the same experimental conditions to determine what fraction, if any, of the observed flux rates are attributable to bacterial growth or other phenomena associated with the tubes. No significant contribution of bacteria to the observed metabolite flux rates was measured in this or prior studies (Girguis et al., 2000). All mass-specific rates are expressed in terms of wet mass.

Effect of varying environmental metabolite concentrations on metabolite flux rates

Sulfide

To determine which chemical species of hydrogen sulfide is taken up by *Riftia* (sulfide, H_2S, or bisulfide, HS^-), as well as the duration of uptake, four *Riftia* weighing 11.9–18.1 g each were placed into two of the high-pressure aquaria immediately after being collected during both the HOT 96 and HOT 97 expeditions (two worms were placed into each vessel). During the HOT 96 expedition, tubeworms were maintained until autotrophic and then ΣH_2S was reduced to 50 μmol l^{-1}, while seawater pH was reduced to 5.66 over a 4 h period. Afterwards, seawater ΣH_2S was increased to 465 μmol l^{-1} over a period of 18 h, at increments of 25–50 μmol l^{-1}. Next, seawater ΣH_2S was again lowered to 50 μmol l^{-1} for 4 h while the pH was increased to 7.4 and seawater ΣCO_2 was increased to 24 mmol l^{-1} (to maintain an equivalent dissolved carbon dioxide concentration) (Childress et al., 1993; Goffredi et al., 1997b). Seawater ΣH_2S was then increased incrementally to 480 μmol l^{-1} over a period of 11 h. During the HOT 97 expedition, tubeworms were maintained until autotrophic and then seawater pH was maintained at 5.8 while ΣH_2S was held at 359.3±8.26 μmol l^{-1}. pH was then increased and maintained at 7.48 over a 4 h period while ΣH_2S was maintained at 346.7±14.16 μmol l^{-1}.

To examine the relation between seawater ΣH_2S concentrations and ΣH_2S uptake, four *Riftia* weighing 13.4–15.1 g were maintained at typical *in situ* conditions for 10 h during the HOT 97 expedition. Seawater ΣH_2S was then increased from 0 to 870 μmol l^{-1} over a period of 12 h, at increments between 50 and 100 μmol l^{-1}.

To determine the duration that chemoautotrophy can be sustained by blood-bound sulfide, three *Riftia* weighing 12.5–14.5 g each were placed into high-pressure aquaria during the HOT 97 expedition (the two smaller worms were placed in one vessel). Seawater ΣH_2S was lowered by decreasing the flow of sulfide gas into the equilibration column. Seawater ΣH_2S was monitored constantly until it decreased to below our level of detection (ca. 5 μmol l^{-1}) (Childress et al., 1984). pH was maintained at 6.1, and all other factors were held at the 'typical' conditions previously described.

To examine the relation between oxygen and ΣH_2S uptake over a range of experimental ΣH_2S concentrations, two experiments were conducted during the HOT97 expedition. In the first experiment, two *Riftia* weighing 12.2–17 g each were placed into the high-pressure respirometers (one worm per chamber) and maintained in 80 μmol l^{-1} ΣH_2S. All other substrates were held at 'typical' concentrations. Next, ΣH_2S was incrementally increased from 80 μmol l^{-1} to 208 μmol l^{-1} over 23 h. In the second experiment, two *Riftia* weighing 9.1–13.6 g each were placed into the high-pressure respirometers (one worm per chamber) and maintained in 200 μmol l^{-1} ΣH_2S. All other substrates were held at 'typical' concentrations. Next, ΣH_2S was incrementally increased from 200 μmol l^{-1} to 843 μmol l^{-1} over 26 h. During both experiments, pH was maintained at 6.1.

Oxygen

To examine the stoichiometric relation between the other major substrates and oxygen uptake, five *Riftia* weighing 7.3–12.1 g each were placed into two of the high-pressure aquaria during the HOT 96 expedition (three worms were placed into one vessel and two worms were placed into the other vessel). Seawater oxygen concentration was increased from 40 to 210 μmol l^{-1} over a period of 23 h, at increments between 15 and 40 μmol l^{-1}. pH was maintained at 5.9, while all other factors were held at the 'typical' conditions previously described.

To determine the duration that chemoautotrophy can be sustained by blood-bound oxygen, three *Riftia* weighing 11.4–14.2 g each were placed into high-pressure aquaria during the HOT 97 expedition. Seawater oxygen was then quickly decreased to below our level of detection by gas chromatography (about 5 μmol l^{-1}) by stopping the flow of oxygen and increasing the flow of N_2 into the equilibration column. pH was maintained at 5.9, while all other factors were maintained at typical *in situ* conditions.

Inorganic carbon

To examine the relation between *Riftia* CO_2 uptake and experimental ΣCO_2 concentrations, three *Riftia* weighing 16–17 g each were placed into high-pressure aquaria during the HOT 96 expedition. Seawater ΣCO_2 was increased from 2.1 to 16.5 mmol l^{-1} over a period of 25 h (at 1 to 2.5 mmol l^{-1} increments) while maintaining pH at 5.9 *via* proportional pH control.

To examine the relation between *Riftia* bicarbonate uptake and experimental ΣCO_2 concentrations, the aforementioned *Riftia* were subject to the same experiment previously described, except that seawater pH was maintained at 6.6 for the duration of the experiment. In both these experiments, all other substrates were maintained at typical *in situ* concentrations.

Temperature

To examine the effects of temperature on *Riftia* host and symbiont metabolism, two experiments were conducted during the HOT 96 and LARVE 98 expeditions. During the HOT 96 experiment, four *Riftia* weighing 10.5–15 g each were placed

into two of the high-pressure aquaria (two worms in each aquaria). Initially, temperature was decreased to 5°C for 7 h, increased to 10°C for 4 h, and then to 20°C for 3 h. During the LARVE 98 expedition, four *Riftia* weighing 12–16 g each were placed in high-pressure aquaria (two in each aquaria). After the onset of autotrophy, temperature was increased to 20°C for 2 h, 27.5°C for 3 h, 30°C for 2 h and 35°C for 2 h.

Individual variation in Riftia metabolite uptake

In order to assess the variation in substrate uptake rates among individual *Riftia* collected from different sites, we collected twelve *Riftia* weighing 12.2–14.1 g, from three different geographical locales, during our HOT 96, HOT 97 and LARVE 98 expeditions. The HOT 96, HOT 97 and LARVE 98 worms were collected from tubeworm clumps located near 12.48N, 103.56W, 9.46N, 104.16W, and 9.50N, 104.17W, respectively, at approximately 2250 m. All worms were collected *via* the *DSV Alvin*, and brought to the surface in a thermally insulated container. All *Riftia* were maintained in our high-pressure respirometry system at typical conditions until autotrophic, during which time metabolite uptake and elimination were recorded for 7 h or more.

Energetics of Riftia symbiont carbon metabolism

To examine the relation between environmental substrate concentration and *Riftia* net carbon fixation (primary productivity), three experiments were conducted during the HOT 97 expedition in which *Riftia* were maintained in different experimental conditions that mimic 'typical', 'better' and 'best' habitats for chemoautotrophic function. In each experiment, four *Riftia* were placed in the high-pressure flow-through aquaria until the onset of autotrophy. Seawater conditions were then adjusted to simulate the conditions at different diffuse flow sites. 'Typical' conditions were ΣCO_2=3.5±0.5 mmol l^{-1}, ΣH_2S=67±12.1 μmol l^{-1}, O_2=97±9.2 μmol l^{-1}, temperature=10°C, NO_3^-=40 μmol l^{-1}, pressure=27.5 MPa. 'Better' conditions were ΣCO_2=4.6±0.7 mmol l^{-1}, ΣH_2S=167±14.2 μmol l^{-1}, O_2=112±7.2 μmol l^{-1}, temperature=15°C, NO_3^-=40 μmol l^{-1}, pressure=27.5 MPa. 'Best' conditions were ΣCO_2=10.8±0.5 mmol l^{-1}, ΣH_2S=256±12.7 μmol l^{-1}, O_2=197±24 μmol l^{-1}, temperature=15°C, NO_3^-=40 μmol l^{-1}, pressure=27.5 MPa. All conditions were maintained for at least 15 h. Metabolite uptake rates recorded after the first 8 h were used to calculate mean metabolite uptake rates. All rates are expressed in terms of wet mass.

Data collection, statistics and plots

Data were collected by Labview 4.0. Rates were calculated using Microsoft Excel, and all statistical analyses and regression plots were rendered on Statview 5.0 (SAS Inc., Cary, NC, USA). 3-dimensional plots were rendered on Transform (Fortner, Inc., Boulder, CO, USA)

Results

Metabolite flux rates and their relation to variations in environmental conditions

Sulfide

Data from the HOT 96 expedition demonstrate that *Riftia* ΣH_2S uptake occurs over a wide range of ΣH_2S concentrations at both pH 5.66 and 7.48 and increases with increasing seawater ΣH_2S concentration (Fig. 1A,B). At pH 5.66, *Riftia's* ΣH_2S uptake rate is more responsive to increasing sulfide concentrations (as seen by the steeper slope in Fig. 1A). At both pH values the relation between ΣH_2S uptake and increasing environmental ΣH_2S concentration appeared linear between 100 and 450 μmol l^{-1} (Fig. 1A,B). In a separate experiment, when pH was maintained at either 5.73 or 7.73 and seawater ΣH_2S concentrations were held constant, *Riftia* ΣH_2S uptake was continuous for over 14 h but there were no significant differences in proton elimination rates or oxygen uptake rates (P>0.05; Spearman correlation and Mann–Whitney U-test, Table 1).

While increasing seawater sulfide concentrations up to 600 μmol l^{-1} stimulated sulfide and oxygen uptake as well as proton elimination (Fig. 2), higher sulfide concentrations resulted in the diminishment of both ΣH_2S and O_2 uptake rates (Fig. 2). During all experiments, ΣH_2S uptake rate correlated

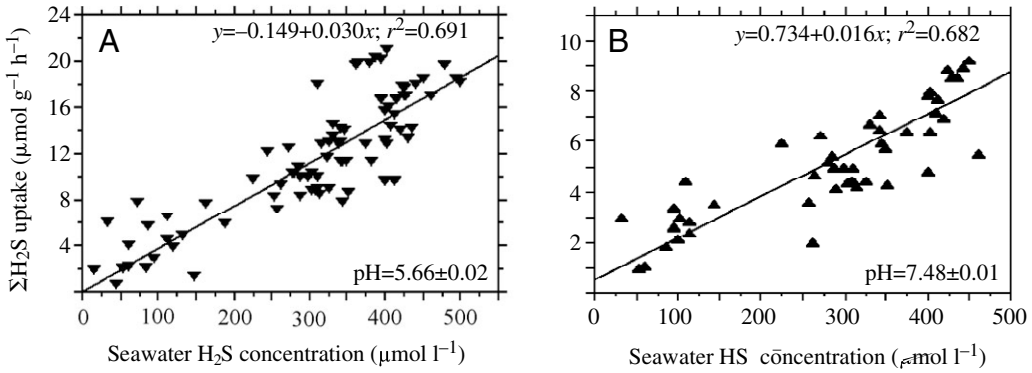

Fig. 1. (A) Plot of ΣH_2S uptake (μmol g^{-1} h^{-1}) as a function of H_2S (μmol l^{-1}) by *Riftia pachyptila* maintained in high-pressure aquaria at pH 5.66. (B) Plot of ΣH_2S uptake (μmol g^{-1} h^{-1}) as a function of HS^- (μmol l^{-1}) by *Riftia pachyptila* maintained in high-pressure aquaria at pH 7.48. All other substrates were held at 'typical' concentrations (see Materials and methods). All rates are expressed in terms of wet mass.

Table 1. *ΣH₂S uptake rate, proton elimination rate and oxygen uptake rate by Riftia pachyptila maintained in two different pH regimes*

pH	ΣH₂S uptake rate (μmol g⁻¹ h⁻¹)	Concentration (μmol l⁻¹)		Proton elimination rate (μequiv. g⁻¹ h⁻¹)	O₂ uptake rate (μmol g⁻¹ h⁻¹)	*N*
		H_2S	HS^-			
5.73±0.07	10.90±0.701	46.23±1.348	313.1±7.528	37.98±7.48	6.62±0.33	49
7.73±0.07	10.23±0.724	317.8±12.92	28.88±1.241	35.2±7.84	5.79±1.34	46

Two *Riftia pachyptila* were placed into two of the high-pressure aquaria, maintained until 'autotrophy' and then kept in two different pH regimes for 18 h and 11 h, respectively. All other substrates were kept at 'typical' *in situ* conditions (O_2=178 μmol l⁻¹, ΣCO_2=4.5 mmol l⁻¹, temperature=15°C, NO_3^-=55 μmol l⁻¹, pressure=27.5 MPa).

Values are means ± s.e.m. *N* = number of measurements in data set. All rates are expressed in terms of wet mass.

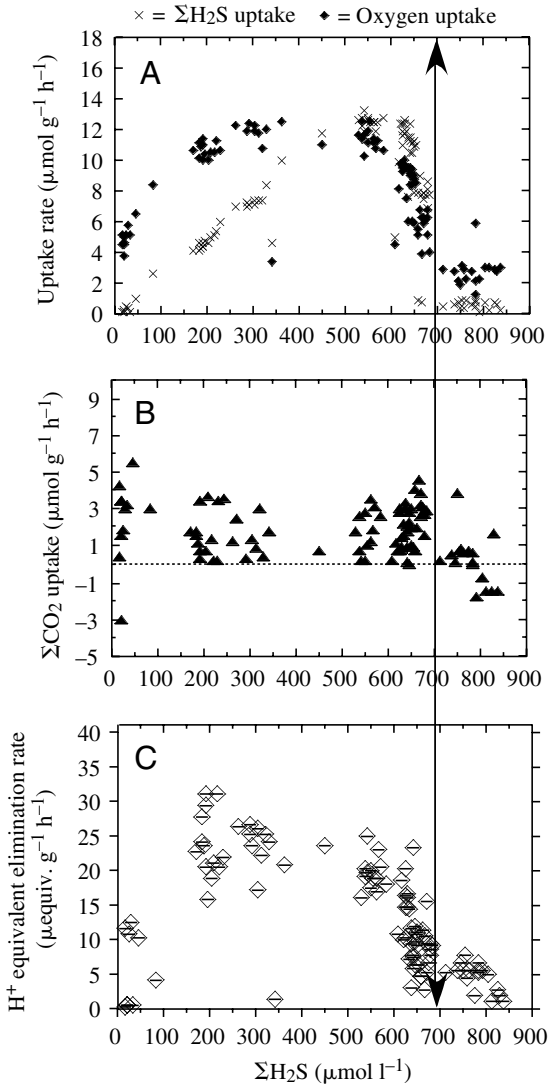

Fig. 2. (A) O_2 and ΣH_2S and (B) ΣCO_2 uptake rates (μmol g⁻¹ h⁻¹); (C) proton elimination rates (μequivalents g⁻¹ h⁻¹), by *Riftia pachyptila* as a function of seawater ΣH_2S (μmol l⁻¹). The large arrow indicates that point at which ΣH_2S was eliminated from the aquarium seawater. pH was maintained at 6.1 and all other substrates were held at 'typical' concentrations (see Materials and methods). All rates are expressed in terms of wet mass.

to oxygen uptake rate (*P*=0.0017; Spearman correlation). ΣCO_2 uptake, however, did not linearly correlate to ΣH_2S or O_2 uptake, and appeared to decrease at higher seawater ΣH_2S concentrations.

When ΣH_2S concentrations were reduced to below the limits of detection (BLD) (Childress et al., 1984), O_2 uptake rates were reduced to 2.88±0.89 μmol g⁻¹ h⁻¹ (Table 2). However, ΣCO_2 uptake was sustained for 5.3 h and O_2 uptake was sustained for 3.5 h (after which O_2 uptake continued at approximately 20% of its original rate; Table 2).

Oxygen

O_2 uptake strongly correlated with seawater oxygen concentration (*P*=0.0001; Spearman correlation; Fig. 3). Total ΣH_2S uptake also strongly correlated with oxygen uptake rate at oxygen concentrations between 50 and 200 μmol l⁻¹ (*P*=0.0001, Spearman correlation; Fig. 3). Proton elimination rate also correlated with seawater oxygen concentration (*P*=0.04; Spearman correlation; Fig. 3). No significant linear correlation was found between ΣCO_2 uptake and O_2 uptake rate (Fig. 3).

In two experiments, the ratio of *Riftia's* O_2 uptake per ΣH_2S uptake was >2 at environmental ΣH_2S concentrations between 100 and 200 μmol l⁻¹ (autotrophic O_2 uptake was determined by subtracting the heterotrophic O_2 uptake rates measured prior to the onset of autotrophy; Fig. 4A). At higher concentrations of environmental ΣH_2S, however, the ratio dropped to <2 (Fig. 4B).

Inorganic carbon

Riftia ΣCO_2 uptake correlated with CO_2 but not HCO_3^- concentrations (Fig. 5). ΣCO_2 uptake appeared to plateau at 8 mmol l⁻¹ CO_2 concentrations, or approximately 16 mmol l⁻¹ total inorganic carbon (Fig. 5) with a maximum ΣCO_2 uptake rate of about 34 μmol g⁻¹ h⁻¹ between 7 and 8 mmol l⁻¹ (Fig. 5). Experiments at higher ΣCO_2 were attempted but not completed due to problems with gas solubility and decreased analytical resolution.

Temperature

Riftia maintained at 5°C had O_2 and ΣH_2S uptake rates of 3.88±0.66 and 1.18±0.73 μmol g⁻¹ h⁻¹, respectively, and net

Table 2. *Data from experiments conducted during the HOT 97 expedition in which either ΣH₂S or oxygen was eliminated from the aquaria seawater containing Riftia pachyptila*

Metabolite	Seawater concentration (μmol l⁻¹) Initial	Final	Time (h) to cessation of ΣCO_2 uptake[a]	Reduction of oxygen uptake	Cessation of ΣH_2S uptake[a]
Sulfide	170	⩽5	5.33	3.50[b]	NA
Oxygen	390	<5	10.5	NA	10.85

Riftia were maintained until they exhibited signs of autotrophy, then either sulfide or oxygen was eliminated from the aquaria seawater. All other substrates were kept at 'typical' *in situ* conditions (see Materials and methods).

[a]Time to cessation indicates the time (h) from when oxygen or ΣH_2S was undetectable in the aquaria water to when uptake ceased.

[b]When sulfide was eliminated from the environment, oxygen uptake was reduced from 14.35±1.23 to 2.88±0.89 μmol g^{-1} wet mass h^{-1}, or 20% of the original rate, reflecting the heterotrophic oxygen demand of the host.

NA, not applicable.

ΣCO_2 production (not uptake) of 1.09±0.80 μmol g^{-1} h^{-1} (Fig. 6). At 10°C ΣCO_2 uptake was 1.75±0.52 μmol g^{-1} h^{-1}. From 10 to 25°C, ΣCO_2, ΣH_2S and O_2 uptake rates increased, with a Q_{10} of approximately 2.3. The sharp increase of ΣCO_2, ΣH_2S and oxygen uptake that occurs at 25°C is a marked departure from the trend at lower temperatures. Optimal temperature for maximal *Riftia* ΣCO_2 uptake is approximately 27°C. Temperatures above 28°C resulted in sublethal reductions in all three measured metabolite uptake rates. Lethal temperature was reached between 30 and 35°C.

The energetics of Riftia symbiont carbon metabolism

Riftia maintained in three different environmental conditions ('typical', 'better' and 'best') exhibited significant differences in metabolite uptake rates as well as proton elimination rates (Table 3). Molar ratios of ΣCO_2 uptake to ΣH_2S uptake ranged from 0.42 at the lower conditions to 1.06 at optimal conditions. Percent energy devoted to carbon fixation was calculated from the energy required to reduce the inorganic carbon to sucrose (–495 kJ mol^{-1}) (Kelly, 1982) and from the energy available from the oxidation of bisulfide to sulfate *via* oxygen

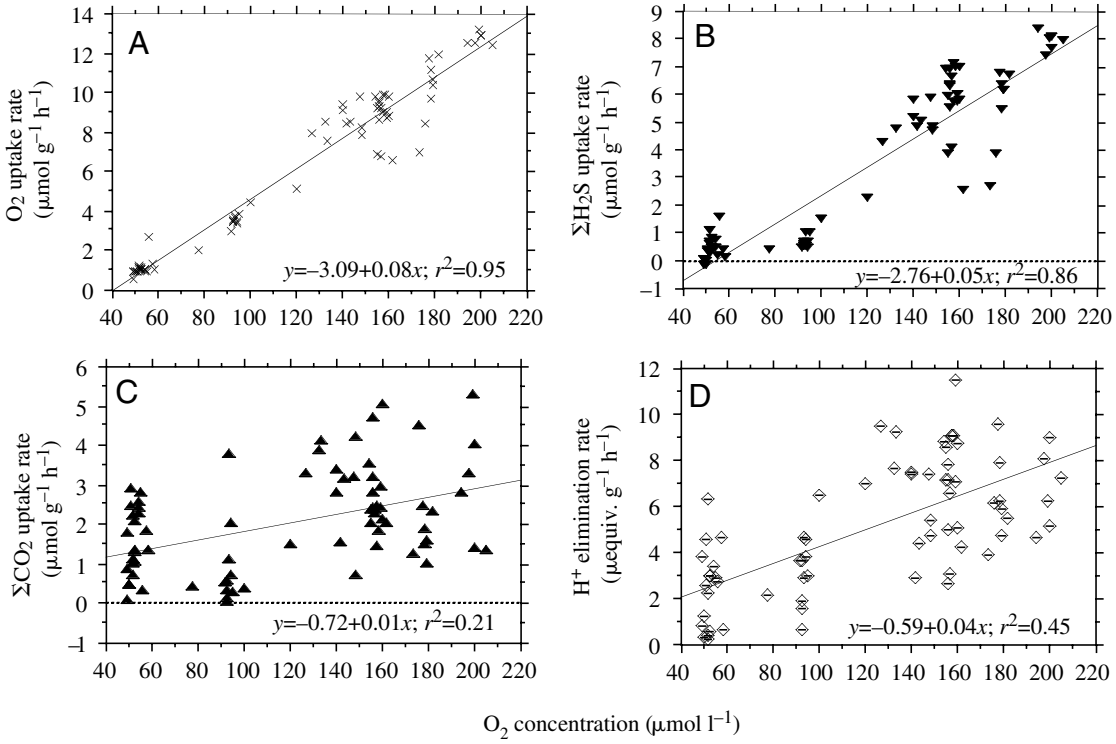

Fig. 3. (A) O_2, (B) ΣH_2S and (C) ΣCO_2 uptake rates (μmol g^{-1} h^{-1}); (D) proton elimination rates (μequivalents g^{-1} h^{-1}), by *Riftia pachyptila* as a function of O_2 concentration (mmol l^{-1}). pH was maintained at 6.1 and all other substrates were held at 'typical' concentrations (see Materials and methods). All rates are expressed in terms of wet mass.

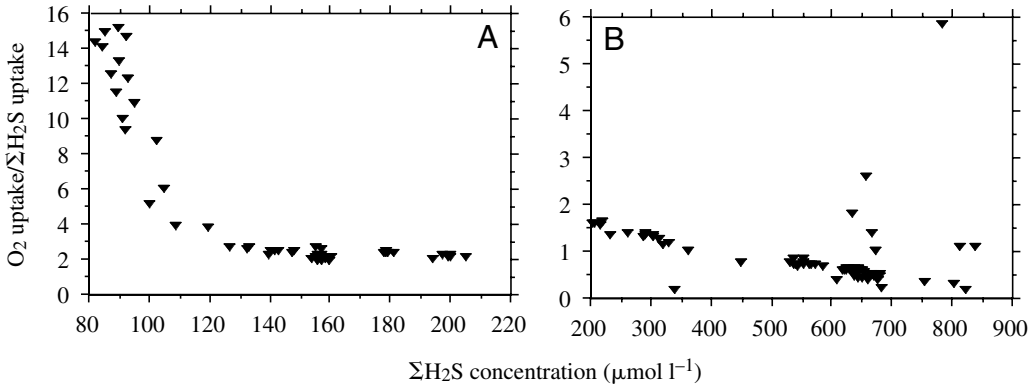

Fig. 4. (A) O_2 uptake:ΣH_2S uptake ratio as a function of ΣH_2S concentration (μmol l^{-1}) by two *Riftia pachyptila* weighing between 12.2 and 17 g each. (B) O_2 uptake:ΣH_2S uptake ratio as a function of O_2 concentration (μmol l^{-1}) by two *Riftia pachyptila* weighing between 9.1 and 13.6 g each. Oxygen uptake rates used to calculate the above ratios have been corrected for heterotrophic contribution (determined from *Riftia* respiration rates prior to autotrophy). pH was maintained at 6.1 and all other substrates were held at 'typical' concentrations (see Materials and methods). All rates were calculated using wet mass.

(–995 kJ mol^{-1}) (Kelly, 1982), and ranged from 21% to 53% at the typical and optimal conditions, respectively.

Variability in Riftia metabolite uptake among individual specimens

No significant differences in ΣH_2S, ΣCO_2, and oxygen uptake rates were observed between the *Riftia* collected from the 'BIOTRANSECT 2' site and the '13 North' site (Table 4). However, *Riftia* collected from the 'BIOTRANSECT 1' site exhibited metabolic uptake and elimination rates that were significantly different from the other two individuals (P=0.0001, Mann–Whitney U-test) and were on average 40–66% lower than the other two individual *Riftia* (Table 4). Significant differences in proton elimination rates were observed among all three *Riftia* tubeworms (Table 4). While there were no superficial differences among the *Riftia*, during subsequent dissections post-experimentation the *Riftia* collected during the LARVE 98 cruise were found to contain blackened trophosome in stark contrast to the green and red trophosomes of the other worms (black trophosomes likely indicate poor symbiont health) (Fisher et al., 1988a).

While we experimented on worms ranging from 4 g to 19 g, this range was not to examine the effects of scaling on metabolic processes.

Discussion

Riftia ΣH_2S and oxygen uptake

Chemosynthetic production depends upon the oxidation of a reduced substrate. In the current experiments, ΣH_2S and O_2 uptake are highly correlated to one another when seawater ΣH_2S and O_2 concentrations are between 10 and 400 μmol l^{-1} (Fig. 3). These concentrations are largely representative of those found *in situ* (Shank et al., 1998; Luther et al., 2001; Mullineaux et al., 2003; Le Bris et al., 2006). Prior studies have shown that symbiont sulfide oxidation is stimulated by oxygen (Fisher and Childress, 1984; Girguis et al., 2000). The data shown here (Fig. 3) demonstrate that symbiont sulfide oxidation is the primary factor influencing *Riftia* oxygen uptake, and is likely responsible for consuming the majority of acquired oxygen. Although nitrate is present at 40 μmol l^{-1} *in situ* and may serve as a terminal electron acceptor for symbiont sulfide oxidation (Hentschel and Felbeck, 1993), a prior study found no correlation between *Riftia* nitrate and ΣH_2S uptake, and that sulfide oxidation cannot be sustained solely by nitrate reduction (Girguis et al., 2000).

Because *Riftia* flourishes in the vent–seawater mixing regimes, simultaneous exposure to both sulfide and oxygen is not likely to be continuous and there may be periods of time in which *Riftia* is not exposed to one substrate or the other (Arndt et al., 1998). However, when we reduced seawater ΣH_2S concentrations to below our level of detection, *Riftia* ΣCO_2 uptake was sustained for 5.3 h, after which *Riftia* exhibited net ΣCO_2 production and decreased oxygen consumption (the remaining oxygen uptake is the host's aerobic respiration; Table 2). We believe this lag time reflects the consumption of hemoglobin-bound sulfide in the vascular and coelomic hemolymph pools (Arp and Childress, 1983; Childress et al., 1984; Zal, 1998). A prior study found that *Riftia* typically consists of 13.9% vascular blood and 19.5% coelomic fluid (Childress et al., 1984), and recent studies have found that vascular and coelomic hemoglobin concentrations in *Riftia* are 3 and 0.5 mmol l^{-1}, respectively (J.J.C., unpublished). Using these values and binding stoichiometries, and assuming that sulfide uptake rates prior to the removal of sulfide reflect symbiont sulfide usage, we estimate that a worm weighing 15 g should have enough bound sulfide to sustain autotrophy for approximately 6 h, a figure comparable to our experimentally determined value.

Although the presence of sulfide is a prerequisite to successful colonization and growth of *Riftia*, exposure to elevated ΣH_2S concentrations *in situ* may be detrimental to

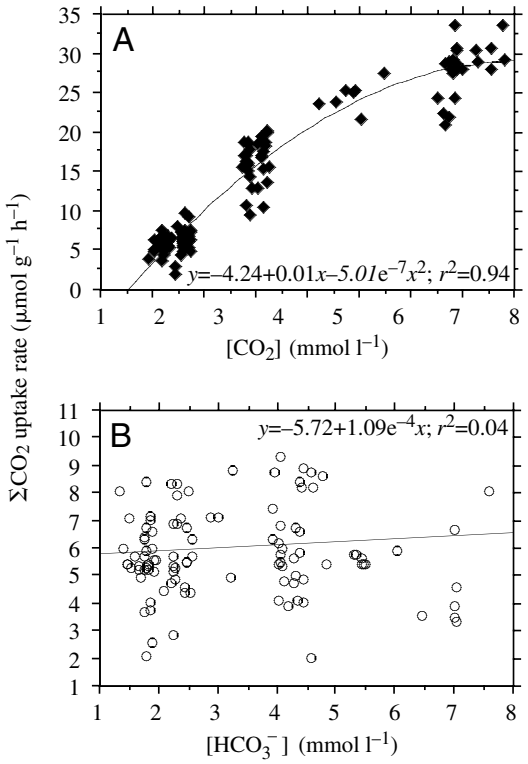

Fig. 5. (A) ΣCO_2 uptake rate (μmol g^{-1} h^{-1}) as a function of CO_2 concentration (mmol l^{-1}) by *Riftia pachyptila* maintained in high-pressure aquaria at pH 5.9. (B) ΣCO_2 uptake rate (μmol g^{-1} h^{-1}) as a function of HCO_3 concentration (mmol l^{-1}) by *Riftia pachyptila* maintained in high-pressure aquaria at pH 6.6. All other substrates were held at 'typical' concentrations (see Materials and methods). All rates are expressed in terms of wet mass.

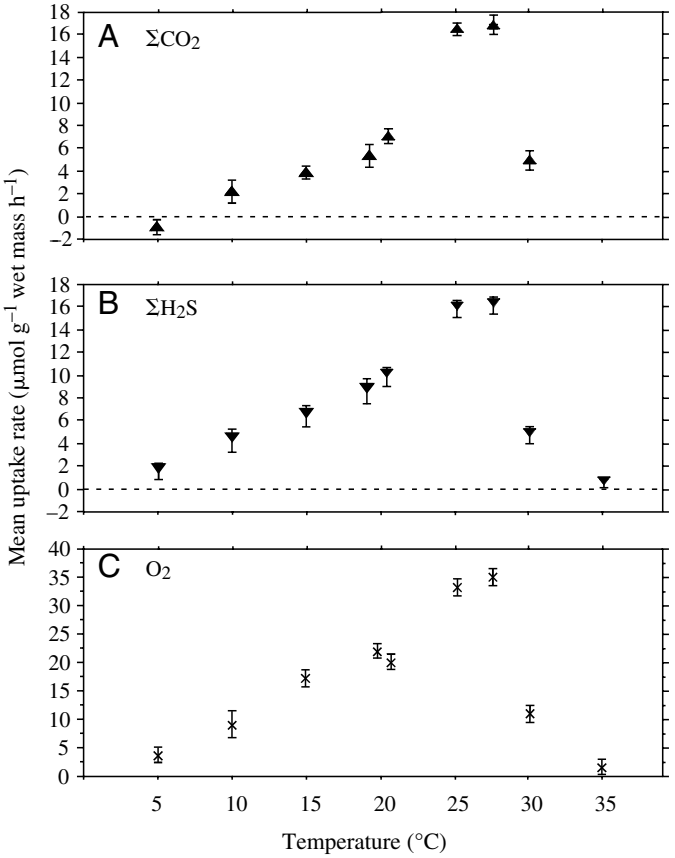

Fig. 6. *Riftia pachyptila* (A) ΣCO_2, (B) ΣH_2S and (C) O_2 uptake rates (μmol g^{-1} h^{-1}) as a function of temperature (°C). The above data represent the results of two separate experiments (5 to 20°C experiment during the HOT 96 expedition, and a 20 to 35°C experiment during the HOT 98 experiment; Data from the HOT 96 expedition are in boldface). Values are means ± s.e.m. (N=4). During these experiments, pH was maintained at 6.0 and all other substrates were held at 'typical' concentrations (see Materials and methods).

Riftia's survival. Our data suggest that inhibition of symbiont metabolism may have occurred after seawater ΣH_2S concentrations reached 700 μmol l^{-1} (Fig. 2). This is higher than the 300 μmol l^{-1} ΣH_2S concentrations that inhibited isolated symbionts in a prior study (Fisher and Childress, 1984). After exposure to high sulfide concentrations, *Riftia's* oxygen uptake rate was comparable to the oxygen uptake rates measured after eliminating sulfide, further suggesting that symbiont sulfide oxidation was diminished. Accordingly, the remaining oxygen uptake likely represents the heterotrophic contribution of the host to total oxygen consumption. While we cannot precisely ascertain the effect of elevated sulfide concentrations on the *Riftia's* aerobic respiration, the similarity to the rates observed in the absence of sulfide suggests that *Riftia* is not prone to sulfide toxicity at 700 μmol l^{-1} seawater ΣH_2S concentrations (Fig. 2). Nevertheless, we observed that all *Riftia* exposed to ΣH_2S concentrations greater than 1.7 mmol l^{-1} in our high-pressure aquaria quickly die. While prior measurements of sulfide concentrations around *Riftia* clumps *in situ* have shown that concentrations vary from 0 to 500 μmol l^{-1} in the water surrounding the worms (Johnson et al., 1988), other studies have measured sulfide concentrations around *Riftia* clumps of approximately 2 mmol l^{-1} (Shank et al., 1998). These observations suggest that *Riftia* may be exposed to higher levels of sulfide *in situ* than previously thought, and may experience symbiont sulfide inhibition *in situ*.

Oxygen inhibition of symbiotic function was not observed to occur at environmentally relevant oxygen concentrations (Fig. 3) even though these are much higher than the concentrations shown to inhibit such function in symbiont preparations (Fisher and Childress, 1984; Fisher et al., 1989; Scott et al., 1994). While these prior studies demonstrated the role of oxygen in sustaining sulfide oxidation by isolated symbionts, they also showed that they are microaerophilic, using oxygen as a terminal electron acceptor in sulfide oxidation but being inhibited at low concentrations of free oxygen. Although no data are available on the free oxygen concentrations (i.e. unbound oxygen) within the bacteriocytes of intact associations, the present data support previous

Table 3. *Data from three experiments conducted during the HOT 97 expedition in which Riftia pachyptila were maintained in three ΣH_2S and oxygen regimes*

Experimental conditions (*N*)	[Sulfide] (μmol l^{-1}) ΣH_2S uptake (μmol g^{-1} h^{-1})	[Oxygen] (μmol l^{-1}) O_2 uptake (μmol g^{-1} h^{-1})	[Inorganic carbon] (μmol l^{-1}) ΣCO_2 uptake (μmol g^{-1} h^{-1})	H^+ equivalent elimination (μequiv. g^{-1} h^{-1})	Mean molar uptake ratio	% Energy devoted to carbon fixation[a]
Typical (26)	67±12.1 3.3±0.5	97±9.2 5.1±0.3	4.5±0.5 1.4±1.1	11.5±2.6	0.42	21
Better (23)	167±14.2 7.8±1.5	112±7.2 12.8±2.2	4.6±0.7 3.7±0.8	23.2±5.7	0.47	24
Best (25)	256±12.7 11.9±0.8	197±24 25.1±0.7	10.8±0.5 12.7±1.1	45.2±9.8	1.06	53

Four freshly-collected *Riftia* were placed into high-pressure aquaria until autotrophy, then seawater substrate concentrations were adjusted to produce 'typical', 'better' and 'best' conditions. Each condition was maintained for at least 15 h, and uptake rates recorded after the first 8 h were used to calculate mean uptake rates (μmol g^{-1} h^{-1}).

Values are means ± s.e.m.; *N*=number of measurements.

Molar ratios were calculated from the ratio of ΣCO_2 uptake per ΣH_2S uptake at 'steady state'.

[a]Percent energy devoted to carbon fixation is calculated from the energy required to reduce DIC to sucrose (–495 kJ mol^{-1}) (Kelly et al., 1982) and the energy available from the oxidation of bisulfide to sulfate *via* oxygen (–995 kJ mol^{-1}) (Kelly et al., 1982).

All rates are expressed in terms of wet mass.

suggestions that free oxygen concentrations within the trophsosome are very low due to the high concentrations of very high oxygen affinity hemoglobins in *Riftia* vascular and coelomic fluids.

Prior studies of *Riftia* have suggested that the species of sulfide acquired by the worm is bisulfide (Goffredi et al., 1997a). In our experiments, *Riftia* sustained similar ΣH_2S uptake rates over a range of environmental ΣH_2S concentrations at both acidic and basic pH values, (ca. 5.5 and ca. 7.6; Fig. 1 and Table 1). At pH 5.5, approximately 99% of the ΣH_2S is hydrogen sulfide (the pK_a is approximately 6.8 at the conditions in our respirometer system). At pH 7.6, approximately 90% of the ΣH_2S is bisulfide. *Riftia's* uptake rates at each pH demonstrate that both H_2S and HS^- can be acquired because the uptake of the minor sulfide species could not support the observed mass-specific ΣH_2S uptake rates. Although *Riftia* may possess mechanisms that reduce the influx of membrane-permeable hydrogen sulfide in order to limit sulfide toxicity (Menon et al., 1995), such a mechanism(s) does not entirely uncouple H_2S uptake and CO_2 uptake by *Riftia*. Rather, it buffers the passive diffusion of hydrogen sulfide into the tissues. However, rapid conversion of H_2S to HS^- within

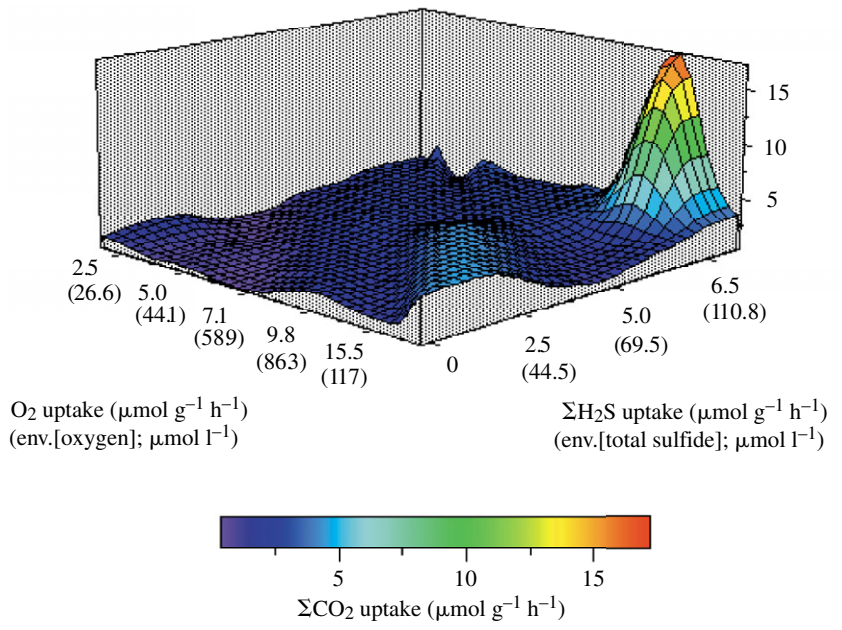

Fig. 7. ΣCO_2 uptake rates (μmol g^{-1} h^{-1}) as a function of O_2 uptake (μmol g^{-1} h^{-1}) and ΣH_2S uptake rates (μmol g^{-1} h^{-1}) by *Riftia pachyptila*. Four *Riftia pachyptila* were placed in high-pressure aquaria, maintained until autotrophy, then O_2 and ΣH_2S concentrations were alternately increased from 27 to 130 μmol l^{-1} and 0 to 140 μmol l^{-1}, respectively. All other substrates were held at 'typical' *in situ* concentrations (see Materials and methods). All rates are expressed in terms of wet mass. Env., environmental.

Table 4. *Substrate flux of three Riftia pachyptila collected from three different sites during our HOT 96, HOT 97 and LARVE 98 expeditions*

Mass of worm (g)	Cruise	Uptake rate (μmol g^{-1} h^{-1}) ΣCO_2	ΣH_2S	O_2	Proton elimination (μequiv. g^{-1} h^{-1})	Collection site	Location
14.35	LARVE 98	4.3±2.1	5.9±0.31	11±0.51	23.6±3.7	BIOTRANSECT 1	9°50′821″N, 104°17.5′83″W
13.58	HOT 97	11.47±3.3*	8.9±2.1*	18.3±1.6*	43.6±5.3	BIOTRANSECT 2	9°46′297″N, 104°16.7′10″W
15.74	HOT 96	15.7±2.8*	7.7±2.6*	16.5±4.3*	31.9±4.5	13 North	12°48′67″N, 103°56′38″W

Tubeworms were maintained in our high-pressure respirometry system at approximately similar conditions (ΣCO_2=4.5–4.8 mmol l^{-1}, ΣH_2S=260–288 μmol l^{-1}, O_2=150–170 μmol l^{-1}, NO_3^-=40–65 μmol l^{-1}, Temperature=15°Celsius, pressure=27.5 MPa).

All worms were collected *via* the *DSV Alvin*, and brought to the surface in a thermally insulated container. Worms were then selected for use in respirometry experiments based on condition (red plumes, no obvious abrasions and appropriate size and length of tube) and maintained until autotrophy. Data are from worms exhibiting autotrophy for 7 h or more.

Values are means ± s.e.m. All rates are expressed in terms of wet mass.

*No significant difference between measurements (within each column; Mann–Whitney *U*-test).

the gill epithelia [where intracellular pH is about 7.4 (Goffredi et al., 1999)] could significantly reduce the risk of mitochondrial sulfide poisoning as HS^- is the species bound by *Riftia's* hemoglobins (Childress et al., 1984; Goffredi et al., 1997a; Flores et al., 2005).

While the precise role of each of these mechanisms remains to be determined, epithelial mitigation of H_2S diffusion, together with the high-affinity HS^- binding hemoglobins, may be the most effective means by which *Riftia* minimizes the effects of sulfide toxicity on host aerobic pathways while maintaining a large pool of sulfide available for symbiont metabolism.

We also observed higher ratios of oxygen uptake to ΣH_2S uptake at lower seawater ΣH_2S concentrations (Fig. 4A). Because the oxidation of sulfide to sulfate stoichiometrically requires 2 O_2 per sulfide, O_2 uptake:ΣH_2S uptake ratios >2 can support the complete oxidation of ΣH_2S to sulfate. This observation supports the prior hypotheses that sulfide oxidation yields sulfate as an end product (Girguis et al., 2002; Goffredi et al., 1997a; Wilmot and Vetter, 1990). However at higher seawater ΣH_2S concentrations, when the ratio dropped to <2 (Fig. 4B), we posit that a fraction of the ΣH_2S may be oxidized to elemental sulfur. Elemental sulfur is commonly found in high concentrations in the trophosome of healthy *Riftia*, and is thought to be a means of storing substrate (Fisher et al., 1988a; Childress et al., 1991). In addition, some fraction of the reductive potential from sulfide oxidation is likely used in inorganic carbon fixation, and that may lead to a shift in the ratio of oxygen uptake to ΣH_2S uptake. Thus, the data shown in Fig. 4 suggest that the end product of sulfide oxidation gradually shifts from sulfate to sulfur at higher seawater ΣH_2S concentrations, and that carbon fixation may increase as the reductive potential from sulfide is more available.

Riftia ΣCO_2 uptake

Our data demonstrate that CO_2 is the chemical species of inorganic carbon that is acquired (Fig. 5), which is consistent with previous *in vitro* and whole animal studies (Childress et al., 1993; Fisher et al., 1988b; Fisher et al., 1990; Goffredi et al., 1997b; Scott, 2003). There is no indication that bicarbonate is acquired, even at higher pH. The ΣCO_2 uptake rate appears highly correlated to environmental CO_2 concentrations (Fig. 5). Because higher environmental CO_2 concentrations would provide a larger gradient and thus more rapid diffusion (Goffredi et al., 1997b), the asymptote of ΣCO_2 uptake rates at 8 mmol l^{-1} environmental carbon dioxide concentrations may reflect a physiological or biochemical limitation in symbiont carbon fixation, although further studies would be required to verify this hypothesis.

Although linear correlations between *Riftia* ΣCO_2 uptake rate and ΣH_2S or oxygen uptake rate were never observed (Fig. 5), our data show that *Riftia* carbon uptake is stimulated by exceeding 'threshold' seawater ΣH_2S and oxygen concentrations (Fig. 7). Future studies should continue to interrogate the relation between energy production (*via* sulfide oxidation) and carbon fixation.

The issue of carbon limitation in *Riftia* has been debated for some time (Fisher et al., 1988b; Fisher et al., 1990; Scott, 2003). While our data show that *Riftia* acquires only 12.5% of the available CO_2 (implying that *Riftia* is not carbon limited), *Riftia's* ΣCO_2 uptake rate consistently responded to increasing seawater ΣCO_2 throughout the duration of the experiment (up to 16 mmol l^{-1} ΣCO_2). However, this observation that *Riftia* ΣCO_2 uptake is, strictly speaking, responsive to changes in seawater ΣCO_2 concentrations does not imply that Riftia is carbon limited. This may be attributable to limitations in another substrate besides DIC. Furthermore, we did not determine if increasing seawater ΣCO_2 concentrations led to biomass accumulation or, alternatively, glycogen accumulation, so the precise relation between increased carbon uptake (and presumably fixation) and growth remains unresolved. This too warrants further investigation.

Temperature effects on Riftia uptake rates

The strongest determinant of metabolite flux, besides limiting substrate concentrations, was temperature. Fig. 6 shows that a sharp increase in ΣCO_2, ΣH_2S and oxygen

uptake occurs at 25°C, a marked departure from the trend at lower temperatures. These data suggest that optimal temperature for maximal *Riftia* uptake, presumably a reflection of symbiont primary productivity, is between 25 and 27°C. Prolonged exposure to temperatures above 32 to 35°C appears to be lethal as all *Riftia* maintained at these temperatures were dead after 2 h. While a prior study suggested that *Riftia* tubeworms were growing rapidly in diffuse vent flows with temperatures ca. 35°C (Shank et al., 1998), diffuse vents are complex thermal regimes and it is unlikely that *Riftia* encounters chronic exposure to these high temperatures. Instead, *Riftia* may tolerate acute exposure to high temperature in order to acquire the sulfide necessary to sustain symbiont autotrophic metabolism. It is notable that *Riftia's* maximal metabolite uptake (and therefore symbiont primary production) occurs at temperatures near their maximal thermal tolerance.

Thermodynamic efficiency

At steady state, when both the experimental conditions and *Riftia* metabolite uptake have remained constant for several hours, ΣCO_2 and ΣH_2S uptake are reliable proxies for carbon fixation and sulfide oxidation rates because they represent the continuous rate of substrate utilization by the symbionts. Accordingly, we determined the mean molar ratios of *Riftia* ΣCO_2 and ΣH_2S uptake to examine the stoichiometric relation between carbon fixation and substrate oxidation by the chemoautotrophic symbionts (Table 3). In our experiments, *Riftia's* ΣCO_2:ΣH_2S uptake ratio varied from 0.42 to approximately 1.06 over a range of environmentally relevant substrate concentrations (Table 3). In a prior study of the bivalve *Solemya reidii*, a clam with chemoautotrophic symbionts in its gill filaments, ΣCO_2 and ΣH_2S uptake molar ratios of 0.86–0.92 were measured (Anderson et al., 1987). These ratios can also be expressed as 'efficiencies', in which the energy utilized in carbon fixation (the conversion of CO_2 to organic carbon) is expressed as a percentage of the total energy available from the oxidation of sulfide to sulphate (Kelly, 1982). *Riftia* efficiencies range from 21% to 53% at 'typical' and 'best' conditions, respectively. In general, it has been observed that more than 80% of the total energy budget of non-hydrogen-oxidizing chemolithotrophs is used in converting carbon dioxide to carbohydrates (Kelly, 1982). The allocation of this energy has been used to explain why the growth yields of chemolithotrophs (already limited by the relatively low molar energy yield of their substrates) are in general rather meager (Kelly, 1990). However, our data demonstrate that *Riftia* symbionts allocate a smaller percentage of their total energy to carbon fixation and nitrate reduction when compared to free-living chemolithotrophic bacteria (Kelly, 1990). This may be attributable to their symbiotic lifestyle since these bacteria do not have to support a myriad of other energy intensive tasks (e.g. spinning flagellae) common among free-living bacteria. These data, as well as the high rates of substrate utilization by *Riftia,* may explain how *Riftia* sustains its rapid growth.

Variability in Riftia metabolite uptake among individual specimens

We observed substantial individual variation in metabolite flux. Under identical experimental conditions, individual *Riftia* exhibited differences in ΣCO_2 uptake that ranged from 4.3 to 15.7 $\mu mol\ g^{-1}\ h^{-1}$ (Table 4). The differences in these carbon uptake rates may reflect the history of the habitat at different collection sites, and those worms with the highest ΣCO_2 uptake rates may have been collected from tubeworm clumps growing atop ample diffuse flow and as such have more metabolically active symbionts. Our observation of blackish trophosomes in the worms with the lowest chemoautotrophic metabolic rates supports this supposition. *In situ* conditions are highly variable and as such can strongly affect *Riftia* symbiont metabolism.

The net effect of environmental conditions on Riftia primary productivity

At steady state, *Riftia* net ΣCO_2 uptake reflects the rate of chemoautotrophic carbon fixation, and can be considered net primary productivity. After placing freshly collected *Riftia* into the high-pressure aquaria and prior to the onset of autotrophy, we measured the response of ΣCO_2 flux to increases in either ΣH_2S or O_2 uptake, and observed no discernable change in ΣCO_2 flux. However, we observed that concomitant increases in both ΣH_2S and O_2 uptake correlated with ΣCO_2 uptake. Specifically, ΣCO_2 uptake drastically increases after seawater ΣH_2S and oxygen concentrations exceed 86 and 95 $\mu mol\ l^{-1}$, respectively (Fig. 7). In every respirometry experiment conducted to date, the onset of autotrophy was preceded by a rapid increase in *Riftia* ΣH_2S and O_2 uptake (enough to consume much of the dissolved metabolite in the aquaria). Whereas in a prior study *Riftia* required ΣH_2S concentrations greater than 90 $\mu mol\ l^{-1}$ to support net carbon fixation (Childress et al., 1991), the current study measured net ΣCO_2 uptake occurring at substantially lower levels of sulfide and oxygen, e.g. 50 $\mu mol\ l^{-1}$ and 70 $\mu mol\ l^{-1}$ respectively (Fig. 3), but only after the threshold ΣH_2S and oxygen concentrations had been exceeded prior to being reduced. The observed phenomenon suggests that (i) carbon fixation is directly mediated by the binding and loading of oxygen and sulfide by *Riftia* hemoglobins or, alternatively, (ii) that *Riftia* (or its symbionts) actively modulates inorganic carbon uptake in response to seawater substrate concentrations, maintaining modest carbon fixation until seawater substrate concentrations are sufficient to support elevated primary productivity. Further studies are required to better address these hypotheses.

In concert, these data demonstrate that *Riftia* metabolite uptake is strongly governed by environmental substrate availability and temperature. *Riftia* symbiont carbon fixation was observed to be highest after sufficient oxygen and sulfide has been acquired by *Riftia*, and when temperatures are relatively high. While the relation between symbiotic function and environmental variability is both facilitated and complicated by the presence of the host, the ultimate constraint on symbiont autotrophic function is the availability of substrates from the environment, and in general *Riftia* is

extremely well-poised to buffer the spatial and temporal variations that are characteristic of diffuse flow regimes. Future studies using longer time-averages of metabolite flux may allow us to develop predictive models of environmental conditions based upon biota observations and, conversely, models of *Riftia* primary productivity based on *in situ* chemical and temperature measurements.

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