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Ana-Voica Bojar, Christophe Lecuyer, William Maher, Hans-Peter Bojar, François Fourel, et al.. Multi-element stable isotope geochemistry and arsenic speciation of hydrothermal vent fauna (*Alviniconcha* sp., *Ifremeria nautili* and *Eochionelasmus ohtai manusensis*), Manus Basin, Papua New Guinea. *Chemosphere*, 2023, 324, pp.138258. 10.1016/j.chemosphere.2023.138258 . hal-04888039

**HAL Id: hal-04888039**

**<https://hal.science/hal-04888039v1>**

Submitted on 15 Jan 2025

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## Multi-element stable isotope geochemistry and arsenic speciation of hydrothermal vent fauna (*Alviniconcha* sp., *Ifremeria nautiliei* and *Eochionelasmus ohtai manusensis*), Manus Basin, Papua New Guinea

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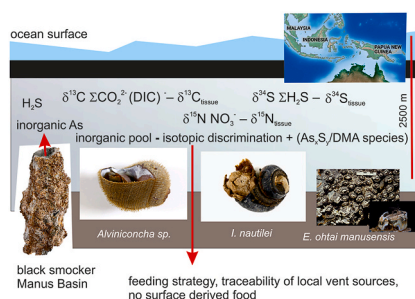
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### HIGHLIGHTS

- Sources of nutrition and survival strategies at the active hydrothermal site Vienna Woods, Manus Basin.
- $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  and element concentrations for the dominant assemblage *Alviniconcha* sp., *I. nautiliei*, *E. ohtai manusensis*
- Insights into the process of metalloid As bioaccumulation and biotransformation in the investigated marine organisms.
- Comparisons with adjacent vent areas of the Manus Basin in order to identify mechanism acting at regional scale.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

Handling Editor: Milena Horvat

#### Keywords:

Stable isotopes  
As speciation  
Metabolic pathways  
Manus basin  
Pacific ocean

### ABSTRACT

Deep-sea hydrothermal vent communities, revealing patterns of niche partitioning, live in a limited area characterised by sharp physico-chemical gradients. In this study, we investigated carbon, sulfur, nitrogen stable isotopes as well as arsenic (As) speciations and concentrations for two snails (*Alviniconcha* sp. and *Ifremeria nautiliei*) and a crustacean, (*Eochionelasmus ohtai manusensis*), occupying distinct niches in the hydrothermal vent field of the Vienna Woods, Manus Basin, Western Pacific.  $\delta^{13}\text{C}$  values of *Alviniconcha* sp. (foot), *I. nautiliei* (foot and chitin) and *E. o. manusensis* (soft tissue) are similar, from  $-28$  to  $-33\text{‰}$  (V-PDB). The  $\delta^{15}\text{N}$  values of *Alviniconcha* sp. (foot and chitin), *I. nautiliei* (foot and chitin) and *E. o. manusensis* (soft tissue) range from 8.4 to 10.6‰. The  $\delta^{34}\text{S}$  values of *Alviniconcha* sp. (foot and chitin), *I. nautiliei* (foot) and *E. o. manusensis* (soft tissue) range from 5.9 to 11.1‰. Using stable isotopes, for the first time, we inferred a Calvin-Benson (RuBisCo) metabolic pathway for *Alviniconcha* sp. along with the presence of  $\gamma$ -Proteobacteria symbionts for the Vienna

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<https://doi.org/10.1016/j.chemosphere.2023.138258>

Received 11 January 2023; Received in revised form 21 February 2023; Accepted 26 February 2023

Available online 9 March 2023

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Woods communities. For *I. nautili*, a feeding pattern is proposed with  $\gamma$ -*Proteobacteria* symbiosis and a Calvin–Benson–Bassham diet with mixotrophic feeding. *E. ohtai manusensis* is filtering bacteria with a CBB feeding strategy, with  $\delta^{15}\text{N}$  values indicating possible higher position in the trophic chain.

Arsenic concentrations in the dry tissue of *Alviniconcha* (foot), *I. nautili* (foot) and *E. o. manusensis* (soft tissue) are high, from 4134 to 8478  $\mu\text{g/g}$ , with inorganic As concentrations of 607, 492 and 104  $\mu\text{g/g}$ , respectively and dimethyl arsenic (DMA) concentrations of 11.12, 0.25 and 11.2  $\mu\text{g/g}$ , respectively. Snails occurring in a vent proximal position have higher As concentration than barnacles, a pattern not observed for S concentrations. Arsenosugars were not put in evidence indicating that the available organic material for the vent organisms are not surface derived.

## 1. Introduction

Only a very small amount of carbon generated by primary production at the sea surface reaches the deep ocean (Suess, 1980). Vent endemic animals depend on the primary production of symbiotic microorganisms, with life cycles driven by chemosynthesis, instead of photosynthesis. Hydrothermal fluids contain  $\text{H}_2$ ,  $\text{CH}_4$ ,  $\text{H}_2\text{S}$ ,  $\text{NO}_3^-$ , As and metal ions, which are used as energy sources for bacteria in order to fulfil their own and host needs (Felbeck et al., 1981; Jannasch et al., 1985; Brooks et al., 1987; Stewart et al., 2005; Nakagawa and Takai, 2008, 2011, McNichol et al., 2016; Le Bris et al., 2019). It has been proposed that the local fluid chemical composition drives the local vent ecology (Luther et al., 2001; Lia et al., 2020) thus symbiosis between chemosynthetic bacteria and invertebrates at deep-sea hydrothermal vents offers opportunities to study the effects of water chemistry, niche segregation and the relationship between symbionts, hosts and diet. An overview of the identified  $\epsilon$ - and  $\gamma$ -*Proteobacteria* symbiotic chemotrophs and associated pathways are given by Yamamoto and Takai, 2011. Since this synthesis work, several new bacteria species have been integrated in the phylogenetic tree of thiotrophic and methanotrophic *Proteobacteria* (Nunoura et al., 2014).

In the Western Pacific, four hydrothermal vent sites, with associated chemosynthetic communities have been described: the Manus Basin, the North Fiji Basin, the Lau Basin and the Mariana Trough. The biological assemblages associated with these vents are different from those occurring in the East Pacific and Mid-Atlantic Ridge. Indeed, in the Western Pacific the prevailing gastropods are primary consumers. Situated close to the fast-spreading rift zone ( $>100$  mm/year) of the Manus Basin, one of the most extended and active hydrothermal fields is Vienna Woods that hosts a faunal assemblage consisting of snails, barnacles, bythograeid crabs, bresiliid shrimps, vestimentiferans, corals and sea anemones (Lisitsyn et al., 1993; Galkin, 1997; Collins et al., 2012). Among the most abundant macro-organisms living at this site, *Alviniconcha hessleri* (Okutani and Ohta, 1988) and *Ifremeria nautili* (Bouchet and Warén, 1991) occupy niches in the immediate vicinity of the vent and *Eochionelasmus ohtai manusensis* (Yamaguchi and Newman, 1990) in the distal position.

*A. hessleri* was the first described hydrothermal vent gastropod with intracellular endosymbiotic bacteria located in its specialized gills (Stein et al., 1988). Endow and Ohta (1989) noted that the apical vacuoles of bacteriocytes contained narrow ducts that directly connect the bacteria to the external medium. *Proteobacteria* have more frequently established endosymbiotic associations with eukaryotes, endosymbionts belonging to four of the five recognized classes, namely the  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\Delta$ . *Alviniconcha* sp. is the first organism known to host distinct bacterial classes, either  $\gamma$ - or  $\epsilon$ - *Proteobacteria* (Suzuki et al., 2005a, b; Urakawa et al., 2005; Suzuki et al., 2006a; Beinart et al., 2012; Breusing et al., 2020b). First described as cryptic (Denis et al., 1993; Kojima et al., 2001; Johnson et al., 2015; Breusing et al., 2020a), *A. hessleri* was refined to six species on the basis of genetic differences. More recently, investigations of the shell morphology of three species, *A. kojimai*, *A. boucheti* and *A. strummeri* indicate non-cryptic differences (Laming et al., 2020). *A. hessleri*, the most investigated species is not described in detail by Laming et al. (2020) and its presence in the Manus Basin, as reported by

Beck (1991) and Galkin (1997) is not discussed in the distribution map of *Alviniconcha* sp. (Fig. 1 in Laming et al., 2020). *Alviniconcha* sp. is distributed at vents along the Central Indian Ridge and the western Pacific in the Manus Basin, Mariana Trough, North Fiji Basin and the Lau Basin (Johnson et al., 2015; Laming et al., 2020).

By examining *I. nautili* specimens collected from the Manus Basin, Galchenko et al. (1993) provided physiological evidence for symbiosis with sulfide-oxidizing bacteria. For a summary of the available descriptions of this species see Bojar et al. (2018). The snail is characterised by hypertrophic gills, a well-developed circulatory system and a reduced stomach, its mobility being much lower than that of *Alviniconcha* sp. A detailed anatomy including distribution of the intracellular, vacuole hosted bacteria in the gill filaments is given by Windoffer and Giere (1997). The animals supply the bacteria with reduced compounds obtained from the vent fluids and with oxygen from the ambient seawater. In turn, bacteria gain energy by oxidizing sulfur species and sometimes methane for the fixation of organic carbon utilized by their hosts (Childress et al., 1986; Cavanaugh et al., 1987; Fisher et al., 1987, 1993; Distel et al., 1995). Desbruyères et al. (1994) confirmed the presence of thiotrophic symbionts by the detection of ribulose-1, 5-bisphosphate carboxylase activity. The type of symbiont described for *I. nautili* is the  $\gamma$ -*Proteobacteria* (Urakawa et al., 2005; Suzuki et al., 2006b). For the Manus Basin, methane-oxidizing bacteria are also present in the gills of the gastropod (Beck and Sobjinski, 1999).

The rims of the hydrothermal vent communities are dominated by the sessile barnacle *E. o. manusensis*. Unlike the mixotrophic diet of the provannids, that are also grazers located next to the autotrophic feeders, the cirripeds from the outer rim of the vent assemblage are filter feeders adapted to the consumption of fine organic particles (Bojar et al., 2018; Ullmann et al., 2018).

Light elements as hydrogen, carbon, nitrogen, oxygen and sulfur have several stable isotopes, which abundances may be measured in phases belonging to organic or inorganic reservoirs. The possibility to determine chemical-physical exchanges between phases and reservoirs of an element makes stable isotope investigations suitable as tracers, for example, for determining the metabolic pathways and diets for terrestrial and aquatic environments. For deep-sea assemblages, the stable carbon, sulfur and nitrogen isotope compositions of soft organic tissue and chitin have been investigated as an indicator of food chain structure by Enright et al. (1981), Brooks et al. (1987), Van Dover and Fry (1989), Colaço et al. (2002), Salerno et al. (2005), De Busserolles et al. (2009), Erickson et al. (2009), Reid et al. (2016), Bojar et al. (2018), Wang et al. (2018), Van Audenhaege et al. (2019), among others. Further investigations have resulted in the identification of isotopic discrimination factors for specific types of endosymbionts or compound specific  $\delta^{13}\text{C}$  values (Suzuki et al., 2005a).

In the present study, in order to determine diets and survival strategies at the active hydrothermal site Vienna Woods, we report  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  data for the dominant assemblage represented by *Alviniconcha* sp., *I. nautili* and *E. ohtai manusensis*. The isotopic measurements applied to soft tissues and shells allow determining the isotopic discrimination between inorganic water compounds used by organism as nutritional sources and symbiont/host itself. The determined isotopic discrimination provides further information into the type of symbiont of

each investigated species and food pathway. A further aim was to provide insights into the process of arsenic (As) bioaccumulation and biotransformation in the investigated marine organisms. As is abundant at hydrothermal vent sites as it is commonly associated with S and can be used by microorganisms that are known to be present in some hydrothermal organisms as symbionts e.g., worms as an alternative energy source (Maher et al., 2016). The acquired data allow making comparisons with adjacent vent areas of the Manus Basin such as Solwara 1 and PACMANUS, in order to identify the mechanisms acting at regional scale and assess sources and pathways of nutrients at the investigated site.

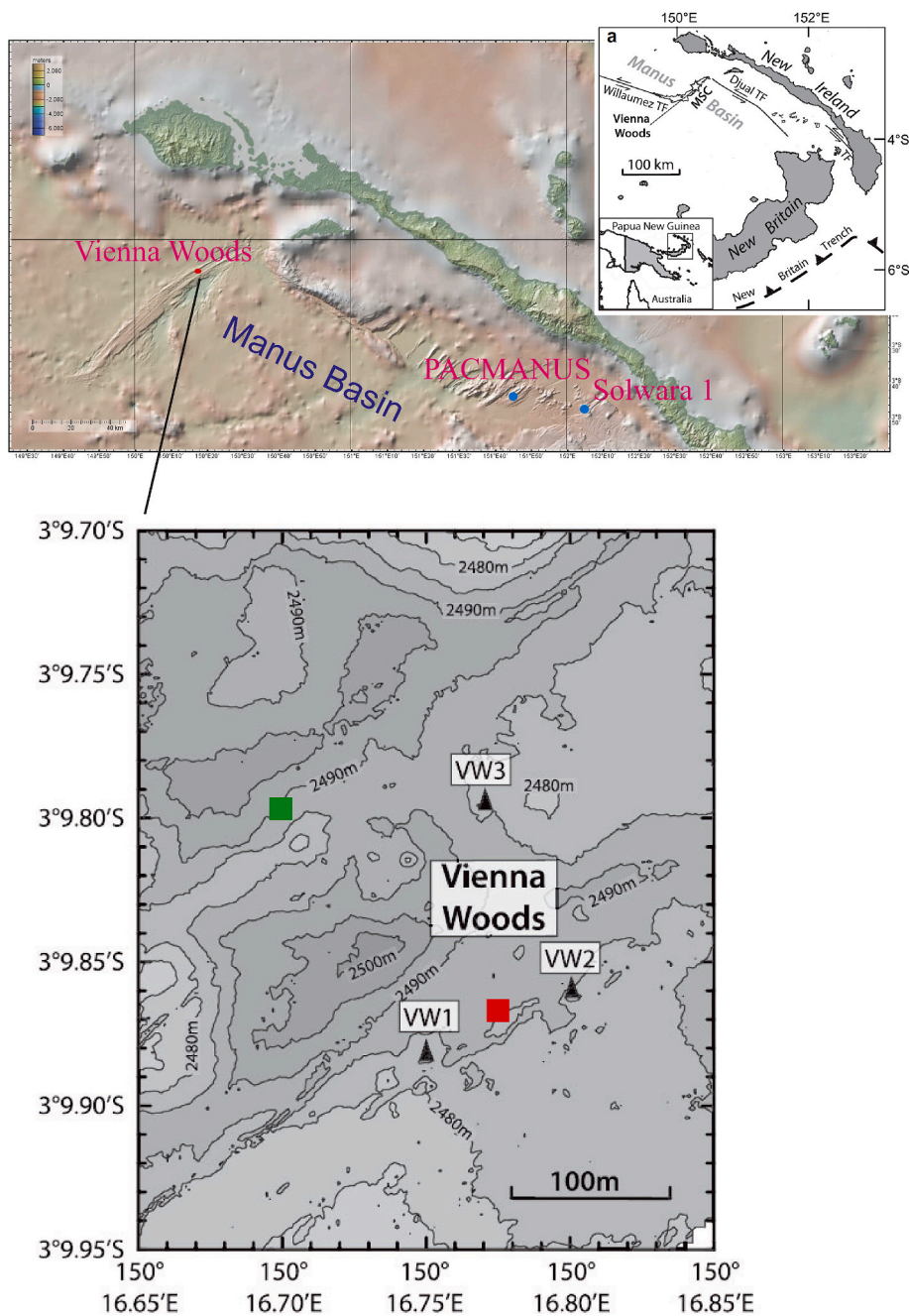
## 2. Material and analytical methods

For this study, the material was collected from the Olga 2 expedition

using equipped ship Sonne (Tufar, 1990). The location of samples from Vienna Woods Manus Basin, which were analysed for this study, is shown in Fig. 1 with a green square. The physicochemical parameters of hydrothermal vents are given in Table 1. The organic material (soft tissues, chitin) was sampled from *A. hessleri* sensu lato, *I. nautili* and *E. ohtai manusensis* as given in Table 2. According to the findings of several authors such as EdwardsTurner and Sharp (2002), Yoshino et al. (2010); Planas et al. (2020), the preservation of sample soft tissues in ethanol allows a safe interpretation of biogeochemical and isotopic data.

### 2.1. Carbon, nitrogen and sulfur stable isotope and concentration analysis

Quantities of 2–4 mg of dry organic material sample were loaded into tin capsules and their  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values were determined using



**Fig. 1.** Geographic setting of the Vienna Woods from the Manus Basin ([www.geomapapp.org](http://www.geomapapp.org)) (see also Table 1) Red square: coordinates for the samples collected for this study; Black triangle: coordinates Reeves et al. (2011); Green square: coordinates for the samples of Suzuki et al. (2006a). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 1**

Location, depth, temperature, geochemistry for the Manus vent sites displayed in Fig. 1. The empty boxes mean that data are not available.

Site	Coord.	Depth (m)	max. T (°C)	pH	ΣH <sub>2</sub> S (mM)/l	H <sub>2</sub> (μM)/l	CH <sub>4</sub> (μM)	ΣCO <sub>2</sub> DIC (mM)/l	δ <sup>13</sup> C <sub>CO2</sub> ‰
This study, sampling 20 Mai 1990, 18 GTVA, Vienna Woods Hydrothermal field 1 ca.1000 m diameter (Tufar and Näser, 1992)	3°9.86'S 150°16.78'E	2500	–	–	–	–	–	–	–
Vienna Woods (Reeves et al., 2011)	3°9.80' to 9.87'S 150°16.75' to 16.80'E	2480–2490	273–285	4.2–5.4	1–1.6	32–55	46–70	3.8–4.47	–4.8 to –5.7
Sampling 20 Oct. 1995, Vienna Woods (Suzuki et al., 2006a)	3°9.8'S 150°16.7'E	2510	–	–	–	–	–	–	–
Sampling January 2006, Solwara 1 (Erickson et al., 2009)	3°47'S 152°5'E	1500	–	–	–	–	–	–	–
Sampling June 2015, Dive 32 PACMANUS (Wang et al., 2018)	3°43.62'S 151°40.32'E	1693	–	–	–	–	–	–	–

**Table 2**

N, C, S stable isotope compositions and N, C, S, element abundance of species from the Manus Basin, Vienna Woods.

Name	δ <sup>15</sup> N ‰	SD ±	n	N% %	SD ±	n	δ <sup>18</sup> O ‰	SD ±	n	δ <sup>13</sup> C ‰	SD ±	n	%C %	SD ±	n	δ <sup>34</sup> S ‰	SD ±	n	%S %	SD ±	n
<i>I. nautiliei</i> (foot)	8.2	0.1	4	11.8	0.1	4	–32.1	0.7	3	50.2	0.3	4	11.1	1.5	3	3.2	0.3	3	0.3	3	
<i>I. nautiliei</i> (chitin)	10.6	0.3	4	15.6	0.4	4	–30.5	0.5	3	50.0	0.9	4	–	–	–	3.1	0.4	3			
<i>I. nautiliei</i> (chitin) <sup>Bojar et al., 2018</sup>							–28.0 to –33.0	0.1	29 serial sampling												
<i>I. nautiliei</i> (calcitic shale) <sup>Bojar et al., 2018</sup>							0.6 to 1	0.1	20 serial sampling	3.1 to 4.6	0.1										
<i>Alviniconcha</i> (foot)	8.4	0.1	4	14.1	0.3	4	–28.5	0.7	3	46.8	0.7	4	9.5	0.4	3	3.2	0.1	3			
<i>Alviniconcha</i> (chitin)	8.9	0.2	4	6.3	0.9	4	–17.3	0.3	3	27.9	2.2	4	8.6	0.7	3	1.1	0.1	3			
<i>Alviniconcha</i> (calcitic shale)							–1.53 to –1.54	0.1	2	4.99 to 4.95	0.1										
<i>E. o. manusensis</i> (whole soft tissue)	9.9	0.1	3	11.1	1.4	4	–32.7	0.3	3	37.5	1.9	3	5.9	0.2	3	8.1	1.8	3			
<i>E. o. manusensis</i> (calcitic shale) <sup>Bojar et al., 2018</sup>							2.4 to 4.3	0.1	60 serial sampling	–0.4 to 1.2	0.1										

<sup>a</sup> Bojar et al. (2018).

an Isoprime100™ isotope ratio mass spectrometer interfaced in continuous flow to a VaryoPyroCUBE™ in combustion mode hosted by the platform “Ecologie Isotopique” of the ‘Laboratoire d’Ecologie des Hydrosystèmes Naturels et Anthropisés’ (LEHNA, UMR 5023, Villeurbanne, France). Carbon, nitrogen and sulfur isotope compositions were calibrated against the following international standards: caffeine IAEA-600 (δ<sup>13</sup>C = –27.77‰ VPDB and δ<sup>15</sup>N = 1.0‰ AIR; Coplen et al., 2006), sucrose IAEA-CH6 (δ<sup>13</sup>C = –10.45‰ VPDB; Coplen et al., 2006), ammonium sulfate IAEA-N2 (δ<sup>15</sup>N = 20.3‰ AIR; Böhlke and Coplen, 1995), silver sulfide IAEA-S1 (δ<sup>34</sup>S = –0.3‰ VCDT; Coplen and Krouse, 1998) and barium sulfate NBS127 (δ<sup>34</sup>S = 20.3‰ VCDT; Halas and Szaran, 2001). C, N and S concentrations (reported in weight%) have been measured by using the traces obtained with the Thermal Conductivity Detector (TCD) of the Elemental Analyzer according to the procedure developed by Fourel et al. (2014). The reference values for concentrations have been calculated from the chemical formula of a rubeanic acid also called dithiooxamide of chemical formula C<sub>2</sub>H<sub>4</sub>N<sub>2</sub>S<sub>2</sub> (Fourel et al., 2014). Standard deviations of analysis are given in Table 2.

## 2.2. Calcite stable isotope analysis

Shell calcite fragments were picked up under binocular and powdered in an agate mortar. Stable isotope ratios of calcite were determined by using an auto sampler MultiPrep™ system coupled to a dual-inlet GV Isoprime™ isotope ratio mass spectrometer (IRMS). For each sample, an aliquot of about 300 μg of calcium carbonate was reacted with anhydrous oversaturated phosphoric acid at 90 °C for 12 min. Carbon and oxygen isotope compositions of calcite are quoted in

the δ notation in per mil relative to VPDB. All samples were measured in duplicate and adjusted to the international references NIST NBS 18 and NBS 19 as well as the in-house standard Carrara Marble. Since 2019 overall reproducibility of the in-house standard Carrara Marble has been ±0.09‰ for δ<sup>18</sup>O (2σ; n = 650) and ±0.06‰ for δ<sup>13</sup>C (2σ; n = 650) with mean δ<sup>18</sup>O and δ<sup>13</sup>C values of –1.04‰ and +2.03‰ (VPDB), respectively. Reproducibility of measured samples is ±0.1‰ for both δ<sup>18</sup>O and δ<sup>13</sup>C values (1σ).

## 2.3. Arsenic measurements

### 2.3.1. Reagents and standards

Nitric acid (HNO<sub>3</sub>; Aristar, BDH) was used for the digestion of samples for determination of the total arsenic concentrations. Ammonium dihydrogen phosphate (Suprapur, Merck) and pyridine (Extra Pure, Merck) were used in the preparation of high-pressure liquid chromatography (HPLC) mobile phases. Formic acid (Extra Pure, Fluka) and ammonia solution (>99.9%, Aldrich) were used for the adjustment of mobile-phase pH. Methanol (HiPerSolv, BDH), acetone, (HPLC, BDH), chloroform (AR, BDH) and deionised water (18.2 MΩ, Sartorius arrium pro) were used for the extraction of arsenic species.

Stock standard solutions (1000 mg l<sup>-1</sup>) of arsenous acid (As<sup>3+</sup>), arsenic acid (As<sup>5+</sup>), monomethylarsonate (MA), and dimethylarsonate (DMA) were prepared by dissolving sodium arsenite, sodium arsenate (DMA) were prepared by dissolving sodium arsenite, sodium arsenate heptahydrate (Ajax Laboratory Chemicals). Disodium methyl arsonate, and sodium dimethylarsenic (Alltech-Specialists) in 0.01 M HCL. Synthetic arsenobetaine (AB) (BCR-626 was obtained from the European Community Institute for Reference Materials and Measurements. Arsenocholine (AC), trimethylarsine oxide (TMAO), dimethylarsinoyl

acetic acid (DMAA), dimethylarsinoylethanol (DMAE), tetramethylarsonium ion (TETRA), trimethylarsoniopropionate (TMAP) and glycerol trimethylated arsonioribose (TriMeOH) were kindly supplied by Kevin Francesconi and Walter Goessler (Institute of Analytical Chemistry, Karl-Franzens-University, Graz, Austria). Glycerol arsenoriboside, sulfonate arsenoriboside and sulfate arsenoriboside (OH-riboside,  $\text{SO}_3$ -riboside,  $\text{OSO}_3$ -riboside respectively) were isolated in house from the marine macroalgae certified reference material *Fucus* (IAEA-140/TM). The phosphate arsenoriboside ( $\text{PO}_4$ -riboside) was isolated in-house from the marine animal certified reference material Oyster tissue1566a (NIST). The identity of these arsenoribosides was previously confirmed by high-performance liquid chromatography-mass spectrometry (HPLC-MS) (Kirby et al., 2004). Thio analogues of MA, arsenosugars, DMAE, DMAA were synthesized by adding a saturated sulfide solution (prepared by bubbling  $\text{H}_2\text{S}$  gas through deionised water for 1 h) to oxo-arsenic standards and reacted for 30 min at a pH of 4. Excess  $\text{H}_2\text{S}$  was removed by purging the solution with argon, then the solutions were evaporated and recrystallised using acetone or dichloromethane. Dimethylthioarsinate (DMTA) was prepared according to Raml et al. (2006). Dimethyldithioarsinate (DMDTA) was prepared in the same way as for DMTA except for pH was adjusted to 6, ethylacetate extraction being excluded. The presence of thio methylated arsenic species were confirmed by mass spectrometry.

#### 2.4. Total arsenic and arsenic species analysis

All extractions were carried out in a nitrogen filled glove bag (Atmos Bags, Sigma Australia). Briefly, using a Intelli-Mixer RM-2 (Elmi, Pty Ltd Riga LV). Homogenised freeze-dried tissues (~0.2 g) were extracted three times with 5 ml of acetone and the supernatant was removed after centrifuging at 4500 rpm for 10 min than supernatants were combined. The acetone was removed, evaporated to dryness using a vacuum concentrator and residues dissolved in 5 ml of 2% v/v nitric acid in a water bath at 90 °C for 60 min in order to analyse the total arsenic content. The pellets from the acetone extraction were then extracted three times with methanol-water (1:1 v/v). Again, the supernatant was removed after centrifuging at 4500 rpm for 10 min and supernatants combined. Supernatants were separated into 5 ml and 10 ml aliquots. Both fractions were evaporated to dryness using a vacuum concentrator and stored in a desiccator until analysis. The pellet of the 5 ml aliquot was redissolved in 5 ml of 1% v/v nitric acid by heating in a water bath at 90 °C for 60 min for total arsenic content analysis while the pellet of the 10 ml aliquot was redissolved in 1 ml of deionised water using sonification for further speciation analysis. The pellet left after the original methanol-water extraction was extracted with 5 ml of 2% v/v nitric acid in a water bath at 90 °C for 60 min. Tissue residues after arsenic species extraction were digested in 2 ml of concentrated nitric acid at 90 °C for 60 min and made up to 5 ml with deionised water.

Total arsenic concentrations in extracts were determined by electrothermal atomic absorption spectroscopy (Deaker and Maher, 1999). The accuracy of total arsenic concentration measurements in organisms, as measured by the sum of arsenic in methanol-water, 2% v/v nitric acid and the residue, was assessed by the analysis of the certified reference material NRCC DORM-2. Measured arsenic concentrations (mean  $\pm$  SD),  $17.5 \pm 0.7$  As  $\mu\text{g/g}$  ( $n = 4$ ) matched the certified value ( $18.0 \pm 1.1$  As  $\mu\text{g/g}$ ). Arsenic species in methanol-water extracts were separated and determined by high-pressure liquid chromatography-inductively coupled plasma mass spectrometry as described by Kirby et al. (2004) and Maher et al. (2013). The chromatographic conditions used were:

1. Arsenic cations: Supelcosil LC-SCX cation exchange column (250 mm  $\times$  4.6 mm, 10  $\mu\text{m}$ ) eluted with a mobile phase containing 20 mM pyridine, pH 2.6 at 1.5 ml  $\text{min}^{-1}$ , 40 °C.
2. Arsenic anions: Hamilton PRP-X200 anion exchange column (250- and 250-mm  $\times$  4.6 mm, 10  $\mu\text{m}$ ) eluted with mobile phases containing

20 mM  $\text{NH}_4\text{H}_2\text{PO}_4$ , pH 3 at 1.0 ml  $\text{min}^{-1}$ , 22 °C and 20 mM  $\text{NH}_4\text{H}_2\text{PO}_4$ , 1%  $\text{CH}_3\text{OH}$ , pH 5.6 at 1.5 ml  $\text{min}^{-1}$ , 40 °C.

3. Thio-arsenic species: Atlantis C18 reverse phase column (150 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ) eluted with a mobile phase containing 20 mM  $\text{NH}_4\text{H}_2\text{PO}_4$ , pH 3 at 1.0 ml  $\text{min}^{-1}$ , 22 °C.

The accuracy of arsenic speciation procedure was determined by the analysis of the certified reference material, DORM-2. The concentrations (mean  $\pm$  SD) of AB ( $17 \pm 1$   $\mu\text{g/g}$ ) and TETRA ( $0.24 \pm 0.01$   $\mu\text{g/g}$ ) measured in DORM-2 tissue ( $n = 3$ ) were similar to certified values (AB,  $16.4 \pm 1.1$   $\mu\text{g/g}$ ; TETRA,  $0.248 \pm 0.054$   $\mu\text{g/g}$ ).

### 3. Results

Data about sampling and physico-chemical measurements at the Vienna Woods already available in the literature are given in Table 1. Table 2 contains the stable isotope compositions and elemental abundances of N, C and S measured in this study for the investigated species. The number and standard deviation of measurements is also shown.

The measured  $\delta^{13}\text{C}$  values of *Alviniconcha* sp. (foot), *I. nautiliei* (foot and chitin) and *E. o. manusensis* (soft tissue) are similar and range from  $-28$  to  $-33\text{‰}$  (V-PDB) with standard deviations ( $\pm 1\sigma$ ) between 0.1 and 0.7 $\text{‰}$ . Carbon element abundances range from 30 to 50% with standard deviations ( $\pm 1\sigma$ ) between 0.3 and 2.2%. The  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of calcitic shales for all investigated specimens are given in Table 2. The  $\delta^{15}\text{N}$  values of *Alviniconcha* sp. (foot and chitin), *I. nautiliei* (foot and chitin) and *E. o. manusensis* (soft tissue) range from 8.4 to 10.6 $\text{‰}$ , with standard deviation between 0.1 and 0.2 $\text{‰}$ . Nitrogen element abundances range from 6.3 to 15.6% with standard deviations ( $\pm 1\sigma$ ) between 0.1 and 1.4%. The  $\delta^{34}\text{S}$  values of *Alviniconcha* sp. (foot and chitin), *I. nautiliei* (foot) and *E. o. manusensis* (soft tissue) range from 5.9 to 11.1 $\text{‰}$ , standard deviations ranging from 0.2 to 1.5 $\text{‰}$ . Sulfur element abundances range from 1.1 to 8.1% with standard deviations ( $\pm 1\sigma$ ) between 0.1 and 0.4%.

Arsenic concentrations for different species and speciation are summarized in Tables 3 and 4. Measured As concentrations in dry tissue for *Alviniconcha* (foot), *I. nautiliei* (foot) and *E. ohtai manusensis* (soft tissue) range from 4134 to 8478  $\mu\text{g/g}$ , with characterised inorganic As concentrations of 607, 492 and 104  $\mu\text{g/g}$ , respectively and DMA arsenic concentrations of 11.12, 0.25 and 11.2  $\mu\text{g/g}$  respectively.

### 4. Discussion

#### 4.1. Temperature distribution at the investigated site and the isotopic composition of DIC

The structure of hydrothermal vent communities is shaped by the ambient temperature, availability of oxygen, dissolved  $\text{CO}_2$  and the availability of reduced compounds ( $\text{H}_2\text{S}$ ,  $\text{H}_2$ ,  $\text{CH}_4$ , As and metals). Using the  $^{18}\text{O}$  isotope thermometer of Kim and O'Neil (1997) for *I. nautiliei* and *E. o. manusensis* calcitic shells, calculated temperatures range from 17 to 21 °C and from 2 to 7 °C, respectively, showing a similar temperature variability of 5–6 °C at the sites where the organisms live (Bojar et al., 2018). *Alviniconcha* sp. is occupying niches similar to *I. nautiliei*, with a more proximal position to the vent sites, in the most  $\text{H}_2\text{S}$ -enriched zone (Pranal et al., 1996; Waite et al., 2008; Childress and Girguis, 2011; Price et al., 2016) but different to the sites occupied by *E. o. manusensis*, therefore the differences in the estimated temperatures. At the low pH values determined around vents (Table 1), the dominant dissolved inorganic carbon species (DIC) is  $\text{CO}_2$ . At an ambient temperature of c. 20 °C, the fractionation between  $\text{CaCO}_3$ – $\text{CO}_2$  aq is 11 $\text{‰}$  (Clark and Fritz, 1997), for an ambient DIC isotopic composition of  $-6\text{‰}$  (Table 1), resulting in a calculated  $\delta^{13}\text{C}_{\text{CaCO}_3}$  value of 5 $\text{‰}$ . The calculated value is similar to the measured ones for the two calcitic snail shells (Table 2). For the barnacles located at a more distal position, we can assume higher pH values closer to marine water, which means in this case a DIC with

**Table 3**Arsenic in extracted fractions (mean  $\pm$  standard deviation, n = 2).

Sample	Organ	Acetone		Methanol		Residue		Total As
		$\mu\text{g/g}$	%	$\mu\text{g/g}$	%	$\mu\text{g/g}$	%	
<i>I. nautili</i>	chitin	8 $\pm$ 2	0.2	148 $\pm$ 34	40.2	213 $\pm$ 12	57.6	371 $\pm$ 65
	foot	9 $\pm$ 1	0.2	598 $\pm$ 12	13.6	3793 $\pm$ 76	86.2	4400 $\pm$ 126
<i>Alviniconcha</i> sp.	foot	8 $\pm$ 1	0.09	1144 $\pm$ 18	13.5	7326 $\pm$ 24	86.4	8478 $\pm$ 42
<i>E. o. manusensis</i>	soft tissue	32 $\pm$ 5	0.77	179 $\pm$ 21	4.3	3923 $\pm$ 50	94.9	4134 $\pm$ 121

**Table 4**

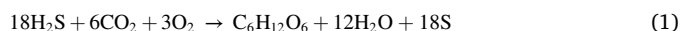
Arsenic speciation-methanol-water extract % (composite sample).

Sample	Organ	Extracted %	Column recovery %	As (III + V) (%)	As(III + V) $\mu\text{g/g}$	MA	DMA (%)	DMA $\mu\text{g/g}$	AB (%)
<i>I. nautili</i>	chitin	41 $\pm$ 1	100 $\pm$ 1	100	151	–	–0.01	–	<0.01
	foot	13.6 $\pm$ 0.3	80 $\pm$ 11	100	492	–	0.05	0.25	<0.01
<i>Alviniconcha</i> sp.	foot	13.5 $\pm$ 0.3	54 $\pm$ 18	98.2	607	–	1.8	11.12	<0.01
<i>E. o. manusensis</i>	soft tissue	4.3 $\pm$ 0.6	65 $\pm$ 11	90.3	104	–	9.7	11.2	<0.01

higher amounts of  $(\text{HCO}_3)^-$  relative to  $\text{CO}_2$ . For the same  $\delta^{13}\text{C}$  value of DIC, the  $\delta^{13}\text{C}_{\text{CaCO}_3}$  value will be lower given that the isotopic fractionation factor  $\text{CaCO}_3\text{-(HCO}_3\text{)}_{\text{aq}}$  is 3.3‰ at 5 °C.

#### 4.2. Carbon and nitrogen stable isotope composition and diet

The vertical distribution of DIC concentrations or  $\Sigma\text{CO}_2$  and  $^{13}\text{C}$  in  $\Sigma\text{CO}_2$  were investigated for the world ocean by Kroopnick et al. (1972); Kroopnick (1985) and references herein. The authors showed that below 1 km the values are almost constant with depths, where DIC concentrations range from 2.2 to 2.1 mM/l with an average  $\delta^{13}\text{C}$  value of 1‰. Vent habitats have higher DIC concentrations and lower  $\delta^{13}\text{C}$  values relative to average seawater. For Vienna Woods, DIC values are between 3.8 and 4.5 mM/l with  $\delta^{13}\text{C}$  of  $-5\text{‰}$  (Table 2). Under oxic conditions, in a process similar to photosynthesis, using  $\text{H}_2\text{S}/\text{H}_2$  and dissolved oxygen instead of light and  $\text{H}_2\text{O}$  (Jannasch and Wirsen, 1979), chemoautotrophic bacteria oxidise  $\text{H}_2\text{S}$  and reduce carbon from DIC resulting in formation of carbohydrates. Symbionts are taking reduced species ( $\text{H}_2\text{S}$ ), DIC and nitrate/ammonia from marine water, with oxygen being provided by the host as follows:



For the investigated species with  $\gamma$ - and  $\epsilon$ -symbionts, there are two major pathways of carbon fixation: the Calvin–Benson–Bassham (CBB) cycle and the reductive tricarboxylic acid (rTCA) cycle (Arnon cycle). The CBB cycle requires three molecules of adenosine triphosphate (ATP) for the fixation of one  $\text{CO}_2$  in contrast to the rTCA cycle requiring one ATP for one  $\text{CO}_2$ . Higher  $\delta^{13}\text{C}$  values are related to the rTCA cycle, with an isotopic discrimination between dissolved DIC and the resulting carbohydrate molecules of  $-2\text{‰}$  to  $-14\text{‰}$ . Lower  $\delta^{13}\text{C}$  values are related to the operation of the CBB cycle with an isotopic discrimination in the range of  $-18\text{‰}$  to  $-33\text{‰}$ . The carbon isotope discrimination during incorporation of  $\text{CO}_2$  in the ribulose bis-phosphate (RuBP) catalyzed by the enzyme ribulose bisphosphate carboxylase-oxygenase (Rubisco) and in the rTCA cycle has been determined a number of times (Park and Epstein, 1960; Hatch and Slack, 1966; Roeske and O'Leary, 1984; Suzuki et al., 2005a), the isotopic discrimination being of a similar magnitude as during incorporation of  $\text{CO}_2$  during C3 and C4 types of photosynthesis.

Suzuki et al. (2005b) measured the carbon isotope composition of *Alviniconcha* sp. tissues ( $\delta^{13}\text{C} = -11\text{‰}$ ) from the Kairei hydrothermal field of the Central Indian Ridge and compared them with the values ( $\delta^{13}\text{C} = -28$  to  $-33\text{‰}$ ) determined for the same species in the south-western Pacific Ocean (Van Dover and Fry, 1989). The values were explained by the presence of different bacteria types in the gill,  $\epsilon$ -for the Indian and  $\gamma$ -for the Pacific Ocean, bacteria processing carbon

through a chemoautotrophy mediated by the rTCA cycle in the first case and the CBB cycle in the second case, with ATP and NADPH generated from sulfur oxidation (Suzuki et al., 2005b). Further studies have shown that *A. hessleri* from the Alice Springs field in the Mariana Back-Arc Basin is also a sulfide-based chemoautotroph organism with  $\gamma$ -*Proteobacteria* (Nakagawa and Takai, 2008; Yamamoto and Takai, 2011). By investigating *Alviniconcha* sp. from the Lau Basin, Beinart et al. (2012) showed that holobionts with  $\epsilon$ -*Proteobacteria* symbionts and higher  $\delta^{13}\text{C}$  values ( $-11.6 \pm 0.4\text{‰}$ ) dominated at fields with higher  $\text{H}_2\text{S}$  concentrations. Holobionts with  $\gamma$ -*Proteobacteria* symbionts and lower  $\delta^{13}\text{C}$  values ( $-27.6 \pm 2.3\text{‰}$ ) were abundant at fields with lower  $\text{H}_2\text{S}$ . Similar low  $\delta^{13}\text{C}$  values (Fig. 2) were measured for *A. kojimai*, in the Manus Basin, Solwara 1 (Erickson et al., 2009), the Lau Basin (Henry et al., 2008) and interpreted as indicating the CBB cycle. Previously, for the PACMANUS site, at fields D and E, Suzuki et al. (2006a) determined at a distance of c. 300 m both populations of *Alviniconcha* sp. with  $\gamma$ - and  $\epsilon$ -*Proteobacteria*. For Vienna Woods, prior to this study, only *Alviniconcha* sp. with  $\epsilon$ -*Proteobacteria* have been described (Suzuki et al., 2006a).

For the samples we investigated, situated c. 300 m apart (Fig. 1) from those of Suzuki et al. (2006a), the low  $\delta^{13}\text{C}$  values of soft tissue and chitin (Table 2) support the presence of the CBB cycle and represent the first determination of *Alviniconcha* sp. with  $\gamma$ -*Proteobacteria* at the site. Interestingly, in the study of Beinart et al. (2012) at the site where  $\epsilon$ -*Proteobacteria* symbionts was put in evidence, the highest  $\Sigma\text{H}_2\text{S}$  median concentration is  $0.239 \text{ mM l}^{-1}$  (Vent field Abe, Table S1, in Beinart et al., 2012), the other sites having lower  $\Sigma\text{H}_2\text{S}$  concentrations. For Vienna Woods, where the investigated species with  $\gamma$ -*Proteobacteria* symbionts were obtained, Reeves et al. (2011) measured  $\Sigma\text{H}_2\text{S}$  of 1.3–1.6 mM/l, much higher than those measured at Lau Basin for sites with  $\epsilon$ -*Proteobacteria* symbionts. These data indicate that  $\Sigma\text{H}_2\text{S}$  concentration is not the limiting factor for the presence of the two endobiont types with different pathways. Probably, carbon isotope discrimination between DIC and host at sites where  $\gamma$ -*Proteobacteria* are operating are related also to DIC concentration, in a similar way as  $\text{CO}_2$  or DIC concentrations determine C3 photosynthesis as shown for example by Farquhar et al. (1989).

Investigations of *I. nautili* (Pranal et al., 1996; Saito and Hashimoto, 2010) and *Alviniconcha* sp. (Pranal et al., 1996) using fatty acid distributions demonstrated that the nutrition of *I. nautili* and *Alviniconcha* sp. relies on bacterial syntheses. For the hydrothermal site situated in the North Fiji Basin, the diet for *Alviniconcha* sp. is mainly based on the sulfur-oxidizing symbiont production in contrast to *I. nautili* with diet contributions from sulfur-oxidizing symbiont production as well as from grazed bacterial mats (Pranal et al., 1996). For the *I. nautili* chitin, Bojar et al. (2018) measured higher  $\delta^{13}\text{C}$  values in the younger stage ( $-31$  to  $-30\text{‰}$ ) and lower in the mature stage, ranging from  $-32$  to  $-33\text{‰}$ . They

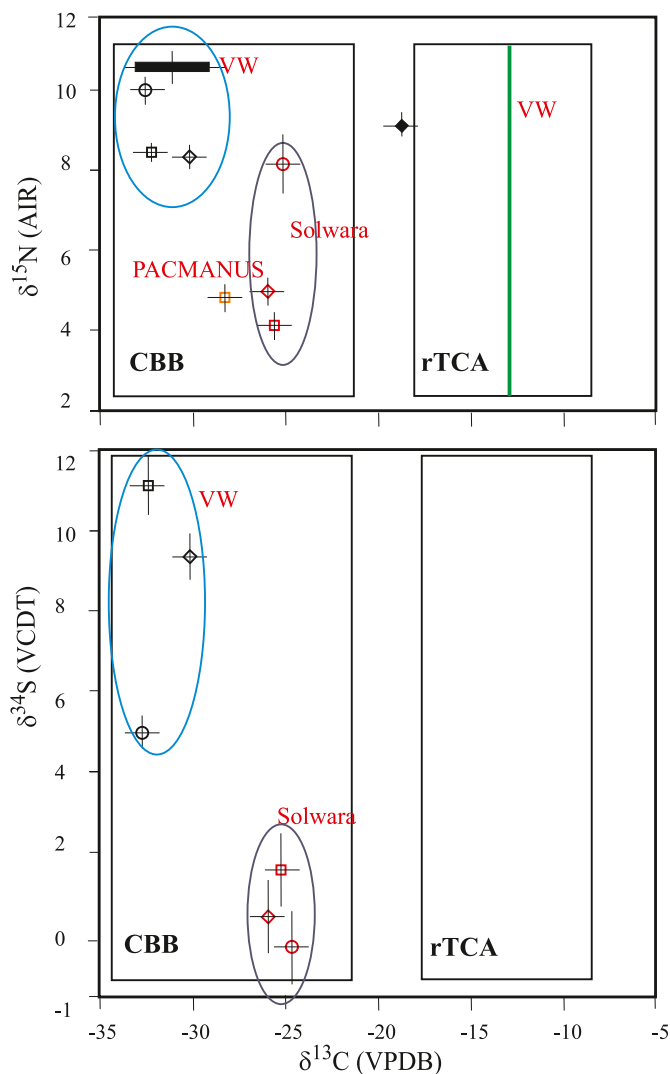


Fig. 2.  $\delta^{13}\text{C}$  -  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  -  $\delta^{34}\text{S}$  biplots for the investigated species. □ *Ifrimeria* foot tissue, ■ *Ifrimeria* chitin, ◇ *Alviniconcha* foot tissue, ◆ *Alviniconcha* chitin, ○ *Eochionelasmus* tissue.

- white and black, this study, Vienna Woods
- green, Suzuki et al. (2006a), only  $\delta^{13}\text{C}$  tissue of *Alviniconcha*, Vienna Woods
- red, Erickson et al. (2009), Solwara 1
- orange, Wang et al. (2018), PACMANUS

The vertical bar for the CBB and rTCA fields were determined using the DIC values of Reeves et al. (2011) and the available maximum and minimum isotopic fractionation for the two cycles.

proposed that the decrease in isotopic compositions may be related to differences in diet strategy: in the younger stage, the  $^{13}\text{C}$ -enriched isotopic composition is related to a more methanotrophic pathway while in the latter stage the thiothrophic pathway becomes predominant. In the light of the study performed by Pranal et al. (1996) who supported the idea of a mixotrophic feeding, the isotopically-based contribution of methanotrophy may be related to a more developed grazing strategy in the younger stage of life, suggesting that the gastropod had a diet using methanotrophic exo- and thiothrophic endo-bacterial inputs. The carbon isotope compositions of *I. nautili* chitin are similar to those measured for foot tissue of  $-32.1\text{‰}$  (Table 2). Similar values were measured for *I. nautili* soft tissue with  $\gamma$ -*Proteobacteria* thiothrophic symbionts from the Lau Basin (Windoffer and Giere, 1997) and for Manus sites as given in Fig. 1 (Erickson et al., 2009; Wang et al., 2018).

Even though the barnacle *E. o. manusensis* is a filter feeder, the

measured carbon isotope composition of its soft tissue is similar to that of the snails. By investigating the Mariana back-arc fluids, Trembath-Reichert et al. (2019) showed that the autotrophic microbial communities (with metabolism based on hydrogen oxidation, denitrification, sulfur/thiosulfate oxidation) are dependent on fluid chemistry, with *Aquificae* bacteria being more abundant in less reducing fluids (low  $\text{H}_2\text{S}$  and  $\text{H}_2$ ) and *Epsilonbacteraeota* being abundant in more reducing fluids (high  $\text{H}_2\text{S}$  and/or  $\text{H}_2$ ). Moreover, in addition to autotrophic bacteria, Meier et al. (2016) also detected heterotrophic *Proteobacteria* showing a zoning habitat at venting areas within the Menez Gwen hydrothermal field, Azores. For several vent sites, including Vienna Woods (Suzuki et al., 2006a), PACMANUS and Desmos caldera (Wang et al., 2017), *Proteobacteria* (including  $\epsilon$ - and  $\gamma$ -*Proteobacteria*) are the dominant exobacteria. In our study, for *E. o. manusensis*, carbon stable isotope composition indicates that the barnacles are filtering autotrophic bacteria using a similar chemoautotrophy as the symbionts, thus nutrition for the barnacle ultimately derives from chemosynthetic sources. The  $^{13}\text{C}$ -depleted signatures are distinct to those of barnacles living offshore (with  $\delta^{13}\text{C}$  ranging between  $-17.5$  and  $-19.7\text{‰}$ , Achituv et al., 1997) with diet based on open-sea phytoplankton (with a  $\delta^{13}\text{C}$  of  $-21\text{‰}$ ), thus photosynthesis.

Symbionts are obtaining  $\text{NO}_3^-$  from seawater that is incorporated into vent organisms. It is documented that there is no appreciable isotopic difference between assimilation of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$  (Hübner, 1986; Fogel and Cifuentes, 1993), nitrate accounting for most of the fixed  $\text{N}_2$  in the ocean (McNichol et al., 2016; Perez-Rodriguez et al., 2017; Sigman and Fripiat, 2018). For the deep ocean,  $\delta^{15}\text{N}_{\text{NO}_3^-}$  values are constant and range from 4 to 6‰ (Peterson and Fry, 1987), nitrogen isotope composition being lighter than at the ocean surface where the heavier values are related to partial consumption of the nitrate by phytoplankton assimilation. For  $\delta^{15}\text{N}$  values, a difference of 3.4‰ indicates a shift between successive trophic levels for consumers with microbial diets (Bergquist et al., 2007), resulting in isotopic compositions of snail tissues similar to those reported in Fig. 2 and Table 2 for the organisms investigated in this study. For the Manus Basin particulate organic matter (POM),  $\delta^{15}\text{N}$  values of 4‰ were measured at a much shallower depth (1600 m) than in the studied case, and interpreted as reflecting the isotopic composition of primary producers, such as chemoautotrophic bacteria. Primary consumers, located on the second trophic level, for example *E. o. manusensis*, show even higher isotopic values than predicted from filtering POM, possible related to a specific bacterial diet or higher trophic level. Erickson et al. (2009) determined a similar trend for the Solwara 1 site (Fig. 1, Table 1) with organisms showing higher  $\delta^{15}\text{N}$  values in the distal position from active vents. The trend is consistent for *E. o. manusensis*, with the most  $^{15}\text{N}$ -enriched isotopic composition recorded for the distal position (Table 2).

The  $\delta^{13}\text{C}$  -  $\delta^{15}\text{N}$  values of investigated barnacle and snails species (Fig. 2) are much lower than for the surface marine domain (for example Achituv et al., 1997; Newsome et al., 2010). This is reflecting the low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the local inorganic deep oceanic pool and the presence of chemosynthesis in the food chain.

#### 4.3. Sulfur stable isotope composition and diet

Total sulfur content (Table 2) is based on dry organic tissues. In the case of *I. nautili*, both foot and chitin contain 3 wt% of S, similar to the values given by Windoffer and Giere (1997) for populations of the Lau basin. *Alviniconcha* sp. contains higher S wt% in foot than in chitin, 3.2 and 1.1 wt% respectively (Table 2), similar to the values determined by Stein et al. (1988) for exemplars of the Mariana Back-Arc Basin. The measured S contents are much higher than the values around 0.5–1% determined for the dry weight of prokaryotic organisms (Zehnder and Zinder, 1980) indicating a systematic enrichment for the investigated vent species.

McDermott et al. (2015) investigated S sources and isotopic equilibrium between dissolved phases and minerals at seafloor hydrothermal



vents, unfortunately the Vienna Wood site is not described in detail.  $\Sigma\text{H}_2\text{S}$  concentrations for Vienna Wood are given in Reeves et al. (2011). At sites with basaltic rocks, the  $\delta^{34}\text{S}$  of sulfide from seawater reflects the isotopic composition of present-day sulfate and host rock sulfide (Shanks et al., 1981; Shanks, 2001). The present-day seawater sulfate has an isotopic composition of 21‰ (Claypool et al., 1980; Longinelli, 1983) with the average  $\delta^{34}\text{S}$  values of sulfides hosted in basalts being  $0.1 \pm 0.5\%$  (Sakai et al., 1984). The mixing of the two pools during reduction-oxidation processes results in seawater  $\Sigma\text{H}_2\text{S}$  with  $\delta^{34}\text{S}$  values comprised between 6 and 7‰ (Shanks, 2001).

According to Childress and Girguis (2011) the two gastropod species with symbionts have gills ranging in weight from 17% to 38% of their wet tissue mass, while for the nonsymbiotic gastropods gills, weights range from 5% to 15%. A schematic diagram representing the physiological function of the two snails having sulfide-oxidizing symbionts is given (Fig. 4, Childress and Girguis, 2011). The net rate (standardized to 20 °C) of metabolic exchange for *Alviniconcha* sp. in comparison with *I. nautiliei* chemoautotrophic symbioses is much higher. The relative body carbon uptake expressed as % body C day<sup>-1</sup> are 6.5 and 0.3%, respectively (Childress and Girguis, 2011). Despite different metabolic rates for carbon- and sulfur intakes (Henry et al., 2008; Childress and Girguis, 2011; Beinart et al., 2015) the  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values are similar for both snails.

Sulfide  $\text{H}_2\text{S}$  is oxidized into  $\text{SO}_4^{2-}$  by symbiotic or free-living bacteria (Gartman et al., 2011). Non-phototrophic bacterial oxidation of  $\text{H}_2\text{S}$  is taking place with variable isotopic discrimination, depending on temperature, concentration and reaction rate (Canfield, 2001). The measured S isotopic composition of *E. o. manusensis*, feeding by filtering is similar with the  $\delta^{34}\text{S}$  values resulted from mixing, indicating minute isotopic fractionation between inorganic  $\Sigma\text{H}_2\text{S}$  and tissues. The  $\delta^{34}\text{S}$  values of snails (Table 1) are a few per mil higher than those measured for *E. o. manusensis*, indicating further isotopic discrimination during  $\gamma$ -bacterial sulfur intake and assimilation. It is known that submarine basalts may be source of sulfur (Wallace and Carmichael, 1992). We suggest that the low  $\delta^{34}\text{S}$  values from all measured tissues are the result of mixing of the dissolved  $\text{SO}_4$  and local volcanic basalt pool with distinct isotopic signature, indicating a local food source with specific isotopic imprint. For active vents, or for active to inactive vents, Erickson et al. (2009) showed that there is no increasing trend for S isotope composition from the proximal to distal positions as is the case of N isotope compositions.

#### 4.4. Arsenic speciation

An additional strategy of gaining energy at vent environments is the oxidation of metals such as Fe (II), Mn, Cu and/or the metalloid As(III) (Nakagawa and Takai, 2008; Maher et al., 2016). For Vienna Woods and PACMANUS, seawater As concentrations are 2  $\mu\text{g/g}$  and in the vent fluids 23–1388  $\mu\text{g/g}$ , respectively (Douville et al., 1999). For Vienna Woods, 70 As concentrations have been measured in sulfide vent ores, four samples having concentrations above 1 wt% while the others are in the order of several hundreds of  $\mu\text{g/g}$  (Lisitsyn et al., 1993). These concentrations are much higher than those documented for sediments located near vents at Desmos Caldera and PACMANUS, where As concentrations range from c. 150–190  $\mu\text{g/g}$  (Wang et al., 2017). The investigated organisms (Table 3) have As concentrations that decrease from the vent (*Alviniconcha* sp.) down to distal positions (*E. o. manusensis*), As in dry mass is 4–8 times the maximal concentrations measured for sulfides. Chitin should not contain As; probably the As in *I. nautiliei* chitin is the result of absorption. The As concentrations measured in organisms in this study are higher than the maximum As concentrations measured by Price et al. (2016) at PACMANUS, for *Alviniconcha* sp. (5580  $\mu\text{g/g}$ ) and for *I. nautiliei* (118  $\mu\text{g/g}$ ). The data suggest that at Vienna Woods, snails are accumulating higher As concentration, which are proportional to the higher As concentrations present in vent solid phases. Wilmont and Vetter (1990) suggest that in hydrothermal vent

worms, the blood system delivers  $\text{S}^{2-}$  and removes waste products such as  $\text{SO}_4$  to protect worms from S toxicity. Entire oxidation is carried out by bacteria and it is likely that similarly, microbial arsenic oxidation helps prevent toxicity. Whether or not the organisms derive energy from this process remains to be determined.

In As speciation analyses, the percentage of As extracted can be low and HPLC column recoveries can be low and variable if the major As are inorganic As forms, for example  $\text{As}_x\text{S}_y$  species, because they do not elute from the column. High column recoveries are achieved if As species like arsenobetaine (AB) are present as they occur in most coastal intertidal/subtidal organisms (Maher et al., 2009). It means in the case of our studied organisms that As is mostly present as inorganic As species, possibly As–S species (Maher et al., 2016). Other species that do not elute include colloidal As (0) but this form is not expected to accumulate in organisms. Beside inorganic As, dimethylarsenic DMA was present at relatively high concentrations for marine organisms (Table 4). In the present case, AB is not present in the investigated organisms as determined by Price et al. (2016) for PACMANUS, for samples collected at depths between 1460 and 1710 m. The most likely source of arsenosugars and AB is from surface derived material such as photosynthetic algae (arsenosugars) and animal detritus (AB). The lack of these As species indicates that organic material available for these vent organisms is not surface derived.

## 5. Conclusions

The investigated fauna, which occupies niches around an active vent, developed different feeding strategies. Similar to the PACMANUS site, *Alviniconcha* shows at a distance of a few hundred of meters, for different fields, symbiosis with  $\epsilon$ - or  $\gamma$ -*Proteobacteria*. For Vienna Woods location, this is representing the first description of *Alviniconcha* sp. symbiosis with  $\gamma$ -*Proteobacteria*. *I. nautiliei* shows a similar depleted carbon isotope composition, supporting also a CBB diet. The  $\delta^{13}\text{C}$  value of chitin suggests mixotrophic feeding, with increased grazing activity in the younger stage organisms. *E. o. manusensis*, a consumer, is filtering bacteria with CBB feeding strategy supported by a similar carbon isotope signature. In the vent distal position,  $\delta^{15}\text{N}$  values are the highest, indicating that *E. o. manusensis* is likely related to a specific bacterial diet or higher trophic level.

Sulfur contents of all investigated species are higher than those of prokaryotic organisms, indicating a systematic enrichment. The isotope compositions determined for *E. o. manusensis* are close to environmental ones, snails show bacterial enrichment during reduction of sulfides. The lower  $\delta^{34}\text{S}$  values of all measured tissues in comparison to marine seawater sulfate is related to mixing with the local volcanic basalt pool with distinct isotopic signature indicating a local food source with specific isotopic imprint.

The low  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values for the local inorganic deep oceanic pool are the starting point of depleted isotopic compositions in comparison to shallow marine species, supporting local recycling of elements in the food web. The lack of arsenosugars also indicates that organic material available for these vent organisms is probably not surface-derived as they are normally produced by photosynthetic algae.

As shown in Fig. 2, probably depending on the concentration of energy delivering phases, for a given association each site has its own isotopic imprint even when the feeding strategy is the same for a given species.

Arsenic as a supplementary energy source shows concentrations in dry tissues higher than those measured for vent mineralization. Snails occurring in vent proximal positions have higher As concentration than barnacles, which is a pattern not observed for S concentrations. Measurable As species are mostly inorganic As with some MA. Accumulation of As, probably occurring as As(III) is used as an energy source by bacterial symbionts, but this hypothesis still needs to be tested.

## Author contributions

Ana-Voica Bojar: Roles/Writing - original draft, figures visualization, sample preparation, Christophe Lécuyer: Writing - review & editing, sample curation, William Maher: Writing - review & editing, sample analysis, Hans-Peter Bojar: Sample curation, sample preparation, François Fourel: Sample analysis, data curation, Ștefan Vasile: Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

All data are published in the paper

## Acknowledgements

We thank three anonymous reviewers for interesting suggestions and remarks which improved our manuscript.

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