Arsenic bioaccumulation and biotransformation in deep-sea hydrothermal vent organisms from the PACMANUS hydrothermal field, Manus Basin, PNG

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ABSTRACT

Deep-sea hydrothermal vents are often enriched in arsenic, and organisms living in these environments may accumulate high concentrations of this and other trace elements. However, very little research to date has focused on understanding arsenic bioaccumulation and biotransformation in marine organisms at deep-sea vent areas; none to date have focused organisms from back-arc spreading centers. We present for the first time concentration and speciation data for As in vent biota from several hydrothermal vent fields in the eastern Manus basin, a back-arc basin vent field located in the Bismark Sea, western Pacific Ocean. The gastropods 
\textit{Alviniconcha hessleri} and 
\textit{Ifremeria nautilei}, and the mussel 
\textit{Bathymodiolus manusensis} were collected from diffuse venting areas where pH was slightly lower (6.2–6.8), and temperature (26.8–10.5 °C) and arsenic concentrations (169.5–44.0 nM) were higher than seawater. In the tissues of these organisms, the highest total measured As concentrations were in the gills of 
\textit{A. hessleri} (5580 mg kg\textsuperscript{-1}), with 721 mg kg\textsuperscript{-1} and 43 mg kg\textsuperscript{-1} in digestive gland and muscle, respectively. \textit{I. nautilei} contained 118 mg kg\textsuperscript{-1} in the gill, 108 mg kg\textsuperscript{-1} in the digestive gland and 22 mg kg\textsuperscript{-1} in the muscle. \textit{B. manusensis} contained 15.7 mg kg\textsuperscript{-1} in the digestive gland, followed by 9.8 mg kg\textsuperscript{-1} and 4.5 mg kg\textsuperscript{-1} in its gill and muscle tissue, respectively. We interpret the decreasing overall total concentrations in each organism as a function of distance from the source of hydrothermally derived As. The high concentration of arsenic in 
\textit{A. hessleri} gills may be associated with elemental sulfur known to occur in this organism as a result of symbiotic microorganisms. Arsenic extracted from freeze-dried 
\textit{A. hessleri} tissue was dominated by As\textsuperscript{III} and As\textsuperscript{V} in the digestive gland (82% and 16%, respectively) and gills (97% As\textsuperscript{III}, 2.3% As\textsuperscript{V}), with only 1.8% and 0.2% arsenobetaine (As-Bet) in the digestive gland and gills, respectively. However, the muscle contained substantial amounts of As-Bet (42% As-Bet compared to 48% As\textsuperscript{III} and 10% As\textsuperscript{V}), suggesting As-Bet is a metabolite. Trace arsenosugar (SO\textsubscript{4}-sug) was observed in digestive gland and gills only. The other snail, \textit{I. nautilei}, was also dominated by As\textsuperscript{III} and As\textsuperscript{V} in digestive glands (82, 10%) and gills (80, 10%), with 6–9% As-Bet, but its muscle contained 62% As-Bet and 32% As\textsuperscript{III}, with 7% trimethylarsoniopropionate (TMAP). Trace dimethylarsinic acid (DMA\textsubscript{V}) was observed in its gills, and trace TMAP and arsenocholine (AC) was observed in digestive glands. The mussel \textit{B. manusensis} was dominated by As-Bet in all three tissue types. Digestive gland and gills contained ~22% As\textsuperscript{III}, 5–10% As\textsuperscript{V}, 20–25% DMA\textsubscript{V}, along with some TMAP and tetramethylarsonium ion (TETRA). However, the muscle contained significantly more As-Bet (91.6%), with the only other species being As\textsuperscript{III} (8.4%). Unfortunately, as is often the case in bioaccumulation and biotransformation studies, extraction efficiencies were low, limiting any rigorous interpretation of arsenic biotransformation patterns. Through process of elimination, we suggest that arsenosugars may be synthesized by H\textsubscript{2}S-oxidizing chemotrophic microbial mats, ultimately leading to the syntheses of As-Bet within vent organisms. However, because As-sugs rarely occur in deep-sea vent organisms, As-Bet, as well as TMAP, AC, and TETRA could also potentially be synthesized directly by the “Edmonds” pathway, the proposed arsensugars to amino acid formation, without the necessity for arsenosugar formation as an intermediate. Future research should endeavor for more comprehensive extraction of organoaarsenicals.

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1. Introduction

Arsenic (As) is a well-known carcinogen and is highly toxic for most organisms. However, many organisms have evolved elaborate mechanisms (e.g., biotransformation to less toxic organoarsenicals, genes that regulate As excretion; Goessler et al., 1997; Oremland and Stolz, 2003; Kunito et al., 2008; Reimer et al., 2010 and references therein; Rahman et al., 2012; Rosen and Liu, 2009; Price et al., 2013a; Zhang et al., 2015), which may allow them to cope with potential toxic effects. Marine organisms in particular have significantly elevated concentrations of As in their tissues (Edmonds and Francesconi, 1987; Cullen and Reimer et al., 1989; Foster et al., 2006). However, most of the arsenic in fish, seaweed, and crustacea is present in the form of non-toxic organoarsenicals.

The two most abundant valence states of arsenic in aqueous systems – from which the organoarsenicals are ultimately synthesized – are the oxidized form arsenate (As(V)), and the reduced form arsenite (As(III); Smedley and Kinniburgh, 2002; Sharma and Sohn, 2009). Marine organisms can take in these inorganic forms of As by two major pathways; cell diffusion from the water column, typically as inorganic arsenic (As(III) is mistaken for PO₄ and brought into the cell via the phosphate transport system, whereas As(V) is taken in via aquaglyceroporins, which are transmembrane channel proteins) and trophic transfer (by eating other organisms containing both inorganic arsenic and previously-synthesized organoarsenicals) (Cullen and Reimer et al., 1989; Sanders et al., 1989; Goessler et al., 1998; Yoo et al., 2004; Rosen and Liu, 2009 and references therein; Duncan et al., 2015; Yang et al., 2012; Maher et al., 2013). Once an organism takes in the inorganic forms of As, a wide range of organoarsenical species can be synthesized. The most abundant and important As species found in marine animals is arsenobetaine (As-Bet, (CH₆)₃As(CH₂COO)³), whereas marine algae typically have arsenosugars (As-sug; a.k.a., arsenoribosides) as the dominant species (Edmonds and Francesconi, 1987). However, a plethora of organoarsenicals are known to exist, with more than 50 characterized to date (Reimer et al., 2010; Kunito et al., 2008). The main forms of inorganic arsenic and synthesized organoarsenicals occurring in marine organisms are presented in Fig. 1.

Often, due to low concentrations, it is difficult to decipher the complex synthesis pathways of organoarsenicals. Targeting organisms surrounding hydrothermal vents can help elucidate these interactions. Arsenic is typically elevated in hydrothermal fluids, and therefore sites where venting occurs are excellent targets for understanding bioaccumulation and biotransformation of arsenic (Price and Pichler, 2005; Breuer and Pichler, 2012; Price et al., 2013a). For example, while seawater concentrations are approximately 26 nM, arsenic concentrations range up to approximately 1.1 μM at the East Pacific Rise (EPR) and 0.3 μM at the Mid-Atlantic Ridge (MAR), and much higher concentrations (up to 18.5 μM) were recorded in the PACMANUS back-arc system (Douville et al., 1999). In the immediate vicinity of deep-sea hydrothermal venting, mostly in the shimmering, lower temperature turbulent mixing zones around the hydrothermal chimneys and diffuse flow vents, unique communities of hydrothermal vent animals thrive. Devoid of sunlight, vent organisms survive by grazing on, and through symbiosis with, microorganisms which gain their energy by oxidizing different reduced compounds like sulfide derived from hydrothermal vent fluids. These microorganisms comprise different species of snails and mussels as well as various crabs, tube worms, and shrimps (Fisher et al., 2007). Studies dealing with As uptake and transformation in marine organisms living at deep sea hydrothermal vent systems are rare, and have to date only focused on MAR systems (e.g., see Larsen et al., 1997; Demina and Galkin, 2008; and Taylor et al., 2012). Furthermore, because As-sugs occur predominantly in photosynthesizing algae, the connection between the formation of As-Bet by As-sug in deep-sea environments seems unlikely. Taylor et al. (2012) showed that animals in deep-sea vent ecosystems contain many of the same organoarsenicals as do pelagic animals, indicating that algae are not the only source of these compounds.

This contribution investigates bioaccumulation of arsenic and the biotransformation patterns of inorganic arsenic to organoarsenicals in vent biota collected from the back-arc spreading center of the Eastern Manus Basin (EMB; Bismark Sea, western South Pacific, Fig. 2). Our results contribute to a broader understanding of arsenic biotransformation in hydrothermal environments, specifically on the potential pathways for synthesis of As-Bet and As-sugs in deep-sea vent organisms. Furthermore, seafloor mining activities will soon commence at hydrothermal sites in the EMB, harvesting sulfide deposits for precious metals (Cu, Zn, Au, Ag) and likely disrupting macro- and microfaunal communities in these sites, as well as potentially influencing metal bioaccumulation due to sulfide particulate production. The results presented here will therefore provide a pre-mining baseline of observations for As bioaccumulation there.

2. Geological setting

Located in the northeastern part of the Bismarck Sea and behind the New Britain Arc-trench system, the Manus Basin is a young (3.5 Ma) and rapidly (137 mm yr⁻¹) opening back-arc basin (Taylor, 1979; Martinez and Taylor, 1996; Tregoning, 2002). It is bordered in the north by the fossil inactive Manus Trench and in the south by the active New Britain Trench, where the Solomon microplate is subducted beneath the New Britain Arc (Fig. 2; Lee and Ruellan, 2006). Active spreading with the formation of new back-arc oceanic crust is currently taking place along three major spreading centers: the western Manus spreading center (WMSC), the 120 km long Manus Ridge spreading center (MSC) erupting MORB-like basaltic lavas (Sinton et al., 2003), and the southeastern ridges (SER).

Hydrothermal venting occurs in the eastern Manus Basin (EMB) in water depths between approximately 1200 and 1700 m as black and white smokers, in addition to discharge of lower temperature clear fluids (Reeves et al., 2011; Thal et al., 2014; Seewald et al., 2015). Several fields in the EMB were sampled during the RV Sonne cruise SO-216 (Bach et al., 2011): including the Papua New Guinea-Australia-Canada-Manus (PACMANUS) hydrothermal system. PACMANUS, discovered in 1991, is located on the 500 m high and 20 km long dacitic to rhyodacitic Pual Ridge in water depths between 1650 and 1740 m (Binns and Scott, 1993; Binns et al., 2007), with massive sulfides observed nearby enriched in Au, Cu, As, Zn, Pb and Sb (Moss and Scott, 2001). Hydrothermal activity was found taking place at five main areas of discrete venting at depths between 1640 and 1710 m, and ranging in size from 50 to 200 m. Roman and Roger’s Ruins, Satanic Mills, and Snowcap discharged focused black smoker fluids from sulfide chimneys, lower temperature grey smokers, and low-temperature diffuse fluid flow through volcanoclastic sediments and cracks near the chimneys (Thal et al., 2014). Erupted lavas consisted of dacite, rhyodacite, and some rhyolite mostly in blocky form (Yang and Scott, 1996). Another new site was found during an expedition in 2006 located ~200 m south of Satanic Mills and in 1710 m water depth. This site was named Fenway, and has a 40 m diameter two-tiered mound in the center made of chimney debris, breccia of anhydrite and sulfide, and coarse anhydrite sand (Reeves et al., 2011; Thal et al., 2014). Compositions of fluids at PACMANUS, as well as those closer to the New Britain Arc at the DESMOS and SuSu Knolls sites, attest to massive outgassing of magmatic SO₂ either at depth within these hydrothermal circulation systems (e.g. at PACMANUS, Reeves et al., 2011) or directly into seawater via fumarolic discharge (e.g. at DESMOS and SuSu Knolls, Gamo et al., 1997; Seewald et al., 2015). At PACMANUS, this outgassing is likely responsible for the highly acidic nature of the black smoker fluids there, which is further enhanced by subsurface mixing processes and associated metal sulfide precipitation (Reeves et al., 2011).
3. Hydrothermal vent fauna from the EMB

Vent-associated biota at EMB are dominantly comprised of different species of gastropods, barnacles, bythograeid crabs, bresiliid shrimps, vestimentiferans, sea anemones, and others (Galkin, 1997). The dominant organisms are snails of the species *Alviniconcha hessleri* and *Ifremeria nautilei* (also described as *Olgaconcha tufari*) from the family Provannidae, in addition to mussels of the species *Bathymodiolus manusensis* (Fig. 3; Galkin, 1997; Desbruyères et al., 2006). The “hairy” yellowish gastropod *A. hessleri* is widespread in the entire western Pacific back-arc basin and the Indian Ocean, namely in the Mariana Trough, the North Fiji, Lau, and Manus Basins (Warén and Bouchet, 2001). This species typically lives in sulfide-rich (up to 750 μM) vent fluids with average temperatures of 10–25 °C. Shells of *A. hessleri* are up to 85 mm in height, and have a reduced digestive tract one-tenth the size of similar species. Within their enlarged gill filaments, which can contain elemental sulfur, these species harbor sulfur-oxidizing thioautotrophic endosymbiotic γ- and ε-proteobacteria (Stein et al., 1988; Suzuki et al., 2005, 2006a). *A. hessleri* relies on these sulfur oxidizers for the bulk of its nutrition, but may also graze on biofilm (Childress et al., 2006).

Compared to *A. hessleri*, specimens of the black snail *I. nautilei* are more widely distributed in the Pacific area, although they have not been found in the Mariana Through. Methanotrophic and sulfur-oxidizing bacteria were found in the gill of these deep-sea provannid gastropods (Galchenko et al., 1992), suggesting that symbiotic bacteria may play a more significant role in the nutrition of this snail. These snails also have a reduced digestive tract, and further investigations indicated that their nutrition is dependent upon endosymbionts that fall phylogenetically into the lineage of the γ-Proteobacteria (Suzuki et al., 2006b).

Mussels of the genus *Bathymodiolus* (*Mytilidae*) are a dominant species in deep-sea hydrothermal vent systems around the world (Van Dover et al., 2002), and their nutrition is also based on chemosynthetic bacterial endosymbionts (Won et al., 2003). At the hydro-
thermal vents in the Manus Basin, *B. manusensis* can gain their energy from the oxidation of reduced compounds present in the hydrothermal fluids (Hashimoto and Furuta, 2007). This process is facilitated by sulfur- and methane-oxidizing bacteria located within their gill epithelial cells in the lateral zone of the gill filaments over which a mixture of hydrothermal fluid and seawater is actively pumped. This pumping is less for filter feeding than to supply H₂S to endosymbionts (Duperron et al., 2009; Duperron, 2010).

All vent organism samples collected in this study were sampled from diffuse vent sites in the PACMANUS hydrothermal field. *A. hessleri* and *B. manusensis* were sampled from an area of low temperature, clear fluid venting near the Fenway area, whereas *I. nautili* was sampled from a low temperature, clear fluid vent area near Roman Ruins.

4. Materials and methods

4.1. Sampling and sample preservation

Mineral precipitation and oxidation are two processes that can change the total As concentration and As species distribution in aqueous fluids immediately after sampling. The most common preservation methods combine filtration, addition of an acid or chelating agent (e.g., HCl, H₂SO₄, or EDTA) to prevent dissolved iron or manganese oxidation and/or precipitation, and elimination of photooxidation by storage in the dark (e.g., Bednar et al., 2004; McCleskey et al., 2004). In sulfidic waters, sample preservation for As is more difficult, because precipitation of poorly crystalline As₂S₃ can substantially lower the amount of total dissolved arsenic (Smieja and Wilkin, 2003). Planer-Friedrich et al. (2007), however, found that flash freezing was the best method for the preservation of arsenic species in sulfidic samples and we therefore flash froze our samples immediately after they were brought onboard. While this approach preserves the As concentration and speciation from that instant on, prior precipitation of iron sulfides in the sample bottles due to cooling during the remainder of the dive or ascent to the surface (~4–8 h) are not accounted for by this method. Because of this problem, we therefore limit our discussion of inorganic arsenic cycling in fluids and assume that measured As concentrations are minimum values that do not represent the vent fluids accurately. The Asfluid data

Fig. 2. Map of the tectonic setting of the Manus back-arc basin showing the major plates and their motions, as well as the different spreading centers and transform faults (WMSC: Western Manus Spreading Center; MSC: Manus Spreading Center; SER: Southeastern Ridges; WIT: Willaumez transform fault; DT: Djual transform fault; WT: Weitin transform fault). (modified from Reeves et al., 2011 and Bach et al., 2011).
reconstituted precipitate (Craddock et al., 2010). However, in the context of the original AsT inventory of the fluids and does not include the reconstituted precipitate "dregs" fraction (Trefry et al., 1994; Craddock et al., 2008; Craddock et al., 2010). However, in the context of bioaccumulation, it is reasonable to assume that vent organisms would have been in contact with higher concentrations than the AsT reported.

Hydrothermal fluid samples from the immediate vicinity of vent organisms were collected for chemical compositions as part of the expedition sampling program using the Kiel Pumping System (KIPS; Garbe-Schönberg et al., 2006). The KIPS was mounted on and deployed from the remotely operated vehicle (ROV) "Quest 4000 m", and made of inert material. Sampling nozzles were made of titanium, and an online temperature probe was installed for in situ temperature measurement during sampling (uncertainty ± 2 °C).

The samples from the KIPS were immediately decanted into PET tubes. Further processing was performed in a glove-bag under an argon atmosphere, where the samples were filtered through an unreactive membrane filter (0.22 µm), and filled into small 4 mL Polystyrels® (HDPE, Zinsser) without headspace. One split from each sample was acidified with sub-boiled HCl for AsT along with another two non-acidified splits for As speciation. After carefully closing the tubes, they were removed from the glove bag and the split for AsT analysis was stored at 4 °C, while the two splits for speciation analysis were immediately frozen at −80 °C. Samples were kept cool or frozen until analysis in the laboratory, where they were thawed shortly before the analyses took place. Several fluid parameters were measured on-board, including pH (at 25 °C, 1 atm). Splits were preserved for major and trace element measurements (these data will be presented in a subsequent manuscript).

4.1. Biota

Several specimens of the snails A. hessleri and I. nautilei, and of the mussel B. manusensis (Fig. 3a-c) were collected with scoop nets manipulated by the mechanical arm of the ROV and transported in temperature isolated storage boxes on the front of the ROV from the seafloor to the surface. Animals were immediately sampled on-board after washing with double deionized water and dissection in a glovebag with argon atmosphere. Each organism was divided into gill, muscle, and digestive gland. Around 20 g wet material from every sample was placed into 20 mL Polystyrels® under argon atmosphere, and then cryofrozen at −80 °C. In the laboratory, samples were freeze-dried, then homogenized with mortar and pestle. Resulting powders were stored at room temperature until further processing.

4.2. Reagents, Standards and Certified Reference Materials (CRM)

All solutions were prepared using double deionized water from a Millipore water purification system (MilliQ Advantage A10, 18 MΩ cm−1), coupled to a Q-POD Element unit. Hydrochloric (HCl) and nitric acid (HNO3) were obtained from a Milestone acid sub-boiling system. Methanol (MeOH) for extraction and hydrogen peroxide (H2O2, 30%) for oxidation experiments were of HPLC and suprapure grade (MERCK), respectively.

Standard stock solutions (1000 mg L−1) for arsenite (AsIII) were prepared from Na2HAsO4·7H2O (Sigma Aldrich) dissolved in 4 g L−1 NaOH (Merck), while arsenate (AsV) was prepared from Na2HAsO3·H2O (Sigma Aldrich) dissolved in water. MAV and DMAV were prepared from (CH3)3AsO(NO3)2·6H2O (Chemservice) and from (CH3)4AsO(NO3)2·6H2O (MERCK), respectively, by dissolving into deionized water. Standards for As-Bet were purchased from Sigma Aldrich and AC from Argus Chemicals, and also dissolved in deionized water. For total As concentration (AsT) measurements, a CertiPur® multi-element standard solution (XVI, MERCK) containing 100 mg L−1 of several other elements in addition to As was used.

Two certified reference materials were used for quality control of the digestion, extraction, and measurement procedures: a dogfish muscle (NRCC-DORM-2), certified for AsT (18.0 ± 0.1 mg kg−1), As-Bet (16.4 ± 1.1 mg kg−1), and the tetramethylarsenion ion (TETRA; 0.248 ± 0.054 mg kg−1), and a tuna fish tissue (BCR-627), also certified for AsT (4.8 ± 0.3 mg kg−1), As-Bet (3.9 ± 0.2 mg kg−1), and DMAV (0.15 ± 0.02 mg kg−1).

Fig. 3. Vent biota in the Manus Basin area sampled during SO-216: (a) the hairy snails Alviniconcha hessleri colonizing the base of a black smoker complex in the Snowcap area, (b) Ifremeria nautilei snails living on the flanks of a black smoker in the Roman Ruins hydrothermal field in low temperature shimmering hydrothermal fluids, and (c) mussel Bathymodiolus manusensis with crabs of Munidopsis lauensis and snails of Ifremeria nautilei. Photos from MARUM®.
For identification of the various As-sug compounds (e.g., phosphate- (PO₄-sug), sulfate- (SO₄-sug), sulfonate- (SO₃-sug), and glycerol-sugar (Gly-sug)), retention times were compared with an aliquot of freeze dried extract of *Fucus serratus* containing these four sugars (Madsen et al., 2000). Algal extracts were provided by Prof. Dr. K.A. Francesconi from the Karl-Franzens University in Graz (Austria).

4.3. Instruments

Sample digestion was performed using a temperature controlled Milestone Ethos digestion system with a microwave power of 1000 W. Concentrations of As⁵⁺ were determined using a Thermo Scientific iCAP Q inductive coupled plasma mass spectrometer (ICP-MS) with an ESI SC-FAST autosampler system. Speciation analyses were performed using a Thermo Scientific ELEMENT 2, high resolution, inductive coupled plasma mass spectrometer (HR-ICP-MS) coupled with an Accela 600 pump and an Accela autosampler system. A Hamilton PRP-X100 column (250×4.1 mm², 10 µm) was used for anion exchange chromatography, both protected with guard columns. Chromatographic conditions are given in Table 1. The ion intensity at m/z 75 (³²As⁺) was monitored in low resolution (LR) and high resolution (HR) modes in order to eliminate the Ar-Cl interference, since the ³⁴Ar⁺ peak can be separated from the ⁴⁰Ar⁺Cl⁺ peak.

4.4. Procedures

4.4.1. Total Arsenic analysis

For measuring As⁤, a Thermo Scientific iCAP Q ICP-MS was used with ¹¹¹In as internal standard (IS). All samples were measured gravimetrically and in triplicate, together with procedure blanks and CRMs. For the different body parts of the mussels and snails, total digestions were performed by weighing in 0.2 g aliquots of freeze-dried, pulverized, and homogenized samples or CRMs (DORM-2 and BCR-627) in digestion vessels and adding 5 mL of concentrated HNO₃ and deionized water to 50 mL. No visible residues were observed in the digestion vessels, indicating complete digestion of tissues.

4.4.2. Arsenic extraction and speciation analysis

For extraction of the different As species from the tissues, 50 mg of each freeze-dried, pulverized, and homogenized sample or CRM material was weighed into 20 mL PTFE tubes. Then, 5 mL of an N₂-purged MeOH/water mixture (1:1, v/v) was added under argon atmosphere and closed before placing them in an end-over-end shaker operating at 20 rpm for 16 h at room temperature. All samples and CRMs were prepared in triplicate. After shaking in the end-over-end shaker, samples were centrifuged for 10 min at 4500 rpm and supernatants were filtered through a 0.22 µm unreactive nylon filter. All samples were stored cool at 4 °C and measured on the HPLC-HR-ICP-MS within hours. The different As species were identified by comparing retention times of the samples with the prepared standards. TMAP and TETRA were identified by comparing with the retention times of the DORM-2 CRM and the different As-sug compounds by comparison with the Fucus serratus extract. Quantification was performed using the calibration curves generated with respective arsenic species eluted nearby. Although DORM-2 is only certified for AB and TETRA, several studies have investigated DORM-2 under very similar chromatographic conditions and have identified and quantified TMAP (Goessler et al., 1998; Francesconi et al., 2000; Kirby and Maher, 2002; Ruiz-Chancho et al., 2013). The results of the present study are in good agreement with the reported concentrations and retention times of the literature for this species. The presence of thio-arsenic compounds was tested by adding hydrogen peroxide (H₂O₂) to the samples to oxidize the thio-arsenic compounds and checking the mass balance of the As species negated their presence.

The total concentration of As in the MeOH extracts was measured using HR-ICP-MS for mass balance calculations and comparison with the sum of the different As species. Results for the measurements of the standards DORM-2 and BCR-627 showed good agreement with the certified values, with extraction efficiency above 90% and an average column recovery of 78% and 86%, respectively.

5. Results and discussion

Nearly 40 years after its first description, many details behind the synthesis of As-Bet are still unclear (Edmonds et al., 1977; Kirby and Maher, 2002), even though many studies have tried to address this issue (Edmonds and Francesconi, 1981, 1987; Cullen and Nelson, 1993; Francesconi and Edmonds, 1993; Francesconi et al., 1999; Francesconi et al., 2000; Madsen et al., 2000; Geiszinger et al., 2002; Edmonds and Francesconi, 2003; Grotti et al., 2008; Taylor et al., 2012; Maher et al., 2013). In deep-sea environments, understanding As-sug synthesis is crucial because it has been postulated that these sugar derivatives are intermediate to the formation of As-Bet (Edmonds and Francesconi, 1981). For example, of the currently 4 known potential, albeit controversial, metabolic pathways for the synthesis of As-Bet in marine organisms, 3 require As-sug as a precursor (Kunito et al., 2008). Generally, the following pathway has been inferred: first, several simple organoarsenicals can be formed following the “Challenger pathway”; As⁵⁺ is taken in by algae and reduced to As³⁺, and further steps are characterized by multioxidative and sequential methylation and reduction steps e.g., As⁵⁺ > As³⁺ > MA⁺ > MA³⁺ > DMA⁺ > DMA³⁺ > TMAO⁺ (Fig. 1, Challenger, 1945, 1955; see also Reimer et al., 2010 for review). Trimethylarsenene gas can be formed microbially, but to form As-sugs, the final reduction and methylation to trimethylarsine gas does not occur. Instead, an adenosyl group is transferred to the arsenic atom by S-adenosylmethionine (SAM), a common methylaing agent in cellular processes (Edmonds and Francesconi, 1987). This compound is adenosine monoarsenenate (Fig. 1), and through this compound we observe not only the exchangeability of arsenate with phosphate in biological systems (e.g., see Wolfe-Simon et al., 2009; Wolfe-Simon et al., 2011), but also we begin to see potential parallels with organoarsenical synthesis and nitrogen transformations within the cell (e.g., adenosine triphosphate; Phillips and Depledge, 1985). At the next step in the pathway, enzymatic, hydrolytic removal of adenine and subsequent steps can then create most of the As-sug compounds observed (Edmonds and Francesconi, 1987). It is thought that arsenosugars can then be converted to As-Bet by several pathways, often with AC as an intermediate (Reimer et al., 2010). Another suspected pathway for the formation of As-Bet is the microbial degradation of As-sugs in
(shallow-sea) marine sediments. This leads to intermediates (e.g., DMAE) which may then be consumed by detritivore and herbivore species, resulting in the synthesis of As-Bet (Edmonds and Francesconi, 1981, 1988; Francesconi and Edmonds, 1993; Goessler et al., 1997; Kirby and Maher, 2002).

One suggested pathway for the synthesis of As-Bet does not require the presence of As-sugs, and could be controlling the accumulation of As-Bet in deep-sea vent organisms (Taylor et al., 2012). This pathway requires only the simple methylated compounds, such as DMAIII produced via the Challenger pathway (Edmonds, 2000). In what we will term the “Edmonds Pathway”, and following the N-As organic compound synthesis analog, DMAIII might replace ammonium ions in the biosynthesis of amino acids, and the arsenylation of pyruvate or glyoxylate could lead then to the synthesis of As-Bet (Edmonds, 2000; Kirby and Maher, 2002). In this case, theoretically one would primarily observe AsIII, AsV, and As-Bet, in tissue extracts (DMAIII is extremely short-lived and unstable in oxidizing environments, and has never been observed in organisms (Kunito et al., 2008)). TMAP can also be formed in this way (Edmonds, 2000; Francesconi et al., 2000; Geiszinger et al., 2002). Because As-Bet, AC, TMAO, and TETRA are arseno-analogues of the nitrogen-containing compounds glycine betaine, choline, trimethylamine oxide, and tetrathylammonium ion, respectively (Shibata and Morita, 1992; Kunito et al., 2008), it is possible the same formation pathway could be true for TETRA and AC. These pathways have yet to be clearly demonstrated.

Previous researchers have shown that marine organisms living in contact with high-As hydrothermal vent fluids can bioaccumulate higher concentrations of this element relative to ‘normal’ marine environments (Neff, 1997; Price and Pichler, 2005; Price et al., 2013b). However, this need not always be the case, because bioaccumulation is a function of not only the amount of As in contact with the organism, but also the physiological makeup (i.e., detoxification efficiency and biosynthesis pathway) of the organism in question (Ruíz-Chañco et al., 2013). Variability in total tissue As concentrations may also be influenced by specimen age (increased time to accumulate more As), and sampling of some organisms with varying degree of direct contact with (i.e., distance from) the hydrothermal plumes (Demina and Galkin, 2008; Price et al., 2013b). Because an organism can take in arsenic both via cell uptake and through the food web, any discussion of arsenic uptake and biosynthesis pathways must also take into consideration the food source of the organism in question. The vent organisms investigated here are known as “diffuse vent organisms”, and are typically found near areas of clear, lower temperature venting. Thus, a primary source of arsenic for these organisms is likely direct uptake from vent fluids (Demina and Galkin, 2008).

Evidence suggests that all three organisms investigated here obtain the bulk of their nutrition through endosymbionts, although their non-vent relatives are either grazers (the gastropods) or filter feeders (the mussels). As a first order assumption, it is possible that the organoarsenicals contained within vent organisms may be synthesized directly from these inorganic forms. If any “ingestion” of organoarsenicals is taking place, it will be through grazing of free-living microbial communities by the gastropods, and through filtration of the water column by the mussels. Previous investigations have shown that A. hessleri can supplement its nutrition through grazing, but I. nautilei and B. manusensis likely do not (Childress et al., 2006). If this is the case, it will be important to understand AsV and As speciation in microbial mat samples (Unfortunately, we did not analyze microbial mats in this investigation). Although it has been speculated that As-sug can be synthesized by the microbial mats (Larsen et al., 1997; Taylor et al., 2012), scrape samples of microbial mats from mid-ocean ridge vents indicate abundant inorganic arsenic but no organoarsenicals were detected (Taylor et al., 2012). Reimer et al. (2010) pointed out that marine snow, which consists of mostly organic matter and includes dead or dying plankton, may provide a source of many arsenicals, including As-sug and As-Bet, to deep-sea organisms. However, deep-sea organisms are thought to be largely chemohautotrophic (Taylor et al., 2012), and it has been shown that only a very small amount of carbon generated by primary production at the sea surface reaches the deep sea (Suess, 1980). As pointed out by Larsen et al. (1997), stable carbon isotopes in the vent shrimp’s muscle tissue has shown that the origin of carbon is non-photosynthetic, indicating that the shrimp grazes on organic material of local origin (Van Dover et al., 1988). Based on these observations, it is very likely that any arsenic taken in by the vent organisms investigated here occurs as the reduced form arsenite, whether that is direct uptake from vent fluids or through injection of microbial mats (Fig. 4).

5.1. As total concentrations

Table 2 presents the temperature, pH, and concentration of arsenic in fluids collected in the vicinity of the organisms sampled for this investigation. A. hessleri was in contact with a warm (Tmax=26.8 °C) fluid with low pH (6.2) and high concentrations of AsV (169.5 nM), relative to seawater (typically 26 nM). I. nautilei was in contact with fluids of Tmax=15.1 °C, a pH of 7.0 and an AsV concentration of 77.4 nM. The mussel B. manusensis was associated with fluids of maximum temperature of 10.5 °C, a pH of 6.8 and an AsV concentration of 44.0 nM (Table 2). Because Fe sulfides within the sampling apparatus precipitate during sampling, these AsV concentrations must be taken as minimums, and we can assume that the organisms encounter higher overall concentrations. Of the three fluids collected, speciation analysis was only conducted for the sample associated with A. hessleri, which revealed approximately equal AsIII and AsV concentrations (71.9 and 71.1 nM, respectively) and an AsIII/AsV ratio of ~1.0. However, as indicated for AsV, these speciation data cannot represent the actual species distribution due to precipitation of Fe sulfides and therefore fluid arsenic speciation will not be discussed further.

Hydrothermal vent animals analyzed in this study accumulate higher concentrations of As, as well as other elements, in their gills, digestive glands, and muscle tissues, at least relative to seawater concentrations (Table 2). The highest concentration of As was found in the snail A. hessleri (6344 ± 14 μg kg−1; Fig. 3a; Table 2), with 5580 ± 14 μg kg−1 in the gill, followed by 721 ± 7 μg kg−1 in the digestive gland and 433 ± 1.5 μg kg−1 in the muscle. For the snail I. nautilei (Fig. 3b), the measurements showed an AsV concentration of 247.6 μg kg−1, with
118.3 ± 1.2 μg kg⁻¹ in the gill, 107.6 ± 1.6 μg kg⁻¹ in the digestive gland and 21.7 ± 0.4 μg kg⁻¹ in the muscle. The concentration of As in the mussel *B. manusensis* (Fig. 3c) reached only up to 30 μg kg⁻¹, with 15.7 ± 0.5 μg kg⁻¹ in the digestive gland, 9.8 ± 0.2 μg kg⁻¹ in the gill, and 4.5 ± 0.1 μg kg⁻¹ in the muscle.

The common approach for evaluating trace metal bioaccumulation in hydrothermal vent organisms is to compare the concentrations with the same organisms collected from a control site unaffected by hydrothermal venting (Price and Pichler, 2005; Price et al., 2013b; Khokhlatiwong et al., 2009). Unfortunately, many deep-sea vent organisms are endemic and therefore do not allow for this comparison. One approach is to compare values known for related organisms, e.g., in shallow-sea environments. For example, bivalves related to *B. manusensis* were shown to contain up to 214 mg kg⁻¹ of As with an average value of 10.44 mg kg⁻¹ (n=151) and marine gastropods related to *A. hessleri* and *I. nautilei* can accumulate between 8.0 and 533 mg kg⁻¹ with average 51.97 mg kg⁻¹ (n=41), with a strong correlation of high As found in marine animals living in environmental polluted areas (Neff, 1997). These values indicate that related organisms can bioaccumulate high concentrations of arsenic overall, but that the vent endemic species can obtain higher concentrations in comparison.

Only a few studies have addressed As bioaccumulation in deep-sea vent organisms. One study indicated that the vent shrimp *Rimicaris exculata* collected from the TAG hydrothermal field contained relatively low concentrations between 4.4 and 21.8 mg kg⁻¹ of As³, in its muscle and gill, respectively. The vent mussel *Bathybiomodiolus puteoserpentis*, collected from the Snakepit hydrothermal field, contained 11.4 and 70.6 mg kg⁻¹ in its muscle and gill, respectively (Larsen et al., 1997). Taylor et al. (2012) focused on *R. exculata* from the TAG and Rainbow vent sites, the mussel *Bathybiomodiolus azoricus* from the Rainbow and Lucky Strike sites, a commensal polychaete found within the mantle of *B. azoricus*, namely *Brachinpolyneopto seepeensis*, and the gastropod *Peltospira smaragdina* from Lucky Strike and TAG sites. As³ concentrations ranged between 3.3 and 29.8 mg kg⁻¹ in *R. exculata*, between 9.9 and 116 mg kg⁻¹ in the mussel, 18.2 mg kg⁻¹ in *B. seepeensis*, and between 14.0 and 67.8 mg kg⁻¹ in the snail *P. smaragdina* (Taylor et al., 2012). Concentrations of arsenic in our vent biota samples were overall much higher than these previously reported values.

Thus, no comparably high values could be found in the literature for snales, either from the deep sea or shallow environments, and 6344 μg kg⁻¹ is to date one of the highest recorded. The extremely elevated As concentrations in the gill of *A. hessleri* might be partly due to the formation of small suspended Fe-S-As particles, which may collect on tissue when the hydrothermal fluid is filtered through the fine filaments of the gill. However, elemental S is also known to occur in the gills of this organism as a result of H₂S oxidation by symbiotic microbes (Childress et al., 2006), and it is possible that the As is associated with this S⁰. Other elements in tissues analyzed here include Cr, Mn, Fe, Co, Cu, Se, Cd, and Pb. Although occasionally their concentrations reflect the As trend (e.g., high amounts of Pb, Cu, and Se found in the gill of *A. hessleri*), the other elements were consistently more elevated in the digestive gland – not the gills – of the different organisms (Table 2). If Fe-S-As particles were responsible for elevated As concentrations, the digestive gland of this organism should be much more elevated in arsenic, given the fact that Fe concentrations are ~5 times higher there compared to the gills. Another piece of evidence that supports the hypothesis that the As is associated with elemental S is the fact that a significant amount of the As³ was released from the gills during the methanol extractions conducted for arsenic speciation analysis (56% or 2580 nM As). Methanol and/or methanol/water at various ratios are useful extractants for organoarsenic compounds, and typically do not affect mineral-bound (i.e., Fe-S-As) arsenic (Francesconi, 2003; Francesconi and Kuehnelt, 2004). Methanol is commonly used to extract S⁰ from marine sediments, and therefore the...
extraction would have potentially released the As bound to S0. We cannot say if part or all of the elevated As concentrations in the gills of A. hessleri are actually bioaccumulated into the gill tissue. As mentioned, in I. nautilei, which also contains elemental S in its gills, arsenic species in each body part followed a very similar pattern to A. hessleri, although not at such high concentrations. We suggest that elemental sulfur present in the gills associated with symbionts is at least partially responsible for the higher concentrations of As in these snails.

5.2. As speciation

Arsenic speciation analysis showed 8 different species in variable proportions for each organism (Tables 3 and 4). In addition to the inorganic AsIII and AsV species, also smaller amounts of DMAV, SO4-sug, As-Bet, TMAP, Ac and TETRA were found. For each of the organisms investigated here, the sum of AsIII, AsV, and As-Bet species made up the majority of As. The incredibly high concentration of AsIII in the gills of A. hessleri suggests a high level of tolerance. If AsIII is actually bioaccumulated in gill tissue, or specifically associated with elemental S, its presence would not necessarily affect cellular function. The digestive gland should be a direct indication of the actual uptake of arsenic, as it functions in much the same way as a mammals liver by absorbing the nutrients (and metals/metalloids) from digested food. AsIII is also the majority species (~82%) in the digestive gland of the A. hessleri, and a similar distribution of As species in general occurs when compared to the gills, albeit with much lower concentrations. Ultimately, any arsenic actually taken up and absorbed by the organism should be metabolized and end up in the muscle tissue, as this is the ‘end’ storage site for any bioaccumulated As. A. hessleri muscle contained 48% AsIII, 42.2% As-Bet, and 9.8% AsV, but none of the other organoarsenicals were extracted. This suggests that As-sug present in trace amounts as SO4-sug in A. hessleri gut and gill may be ingested, rather than metabolized.

The distribution of arsenic species in I. nautilei was very similar to A. hessleri, except that no As-sugs were identified. Arsenite again had the highest enrichment in both the gill and digestive gland for this organism, although concentrations were not nearly as elevated. These lower concentrations are likely due to the fact that this organism is in less direct contact with high-As fluids, and further suggests an uptake pathway is via absorption from vent fluids. Uptake and accumulation pathways may be similar to A. hessleri, given that these are closely related organisms (i.e., both of the Family Provaniidae). I. nautilei As speciation patterns were dominated by AsIII and AsV in digestive glands (82, 10%) and gills (80, 10%), with 6–9% As-Bet, but its muscle contained 62% As-Bet and 32% AsIII, with 7% TMAP. Trace DMAV was observed in its gills, and trace TMAP and AC was observed in digestive glands. These As species can be intermediates in the formation of As-Bet, as indicated above.

The arsenic speciation pattern in B. manusensis is somewhat different, although not at such high concentrations. We suggest that elemental sulfur present in the gills associated with symbionts is at least partially responsible for the higher concentrations of As in these snails.

Table 3
Average arsenic species concentrations (n=3), for the two snails A. hessleri and I. nautilei and the mussel B. manusensis compared to two CRMs.

<table>
<thead>
<tr>
<th>Vent animal/Ref material organ</th>
<th>Extraction efficiency %</th>
<th>Colum recovery %</th>
<th>AsIII mg kg⁻¹</th>
<th>AsV mg kg⁻¹</th>
<th>DMA mg kg⁻¹</th>
<th>SO4-sug mg kg⁻¹</th>
<th>As-Bet mg kg⁻¹</th>
<th>TMAP mg kg⁻¹</th>
<th>Ac mg kg⁻¹</th>
<th>TETRA mg kg⁻¹</th>
<th>Σ SPECIES mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alviniconcha hessleri</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>DG</td>
<td>30.1</td>
<td>86.4</td>
<td>153.2</td>
<td>29.4</td>
<td>1.4</td>
<td>3.5</td>
<td>187.5</td>
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<tr>
<td>Gill</td>
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<td>84.7</td>
<td>2580.4</td>
<td>60.1</td>
<td>6.7</td>
<td>4.0</td>
<td>2651.2</td>
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<tr>
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<td>58.3</td>
<td>38.4</td>
<td>4.7</td>
<td>1.0</td>
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<td>4.1</td>
<td>9.7</td>
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<td>11.6</td>
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</tr>
<tr>
<td>DG</td>
<td>20.9</td>
<td>97.6</td>
<td>18.0</td>
<td>2.2</td>
<td>1.3</td>
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<td>0.2</td>
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<tr>
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<td>23.9</td>
<td>3.0</td>
<td>0.3</td>
<td>2.6</td>
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<tr>
<td>Muscle</td>
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<td>0.9</td>
<td>0.1</td>
<td>1.8</td>
<td>0.2</td>
<td>3.0</td>
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<td>42.8</td>
<td>5.2</td>
<td>0.3</td>
<td>5.8</td>
<td>0.4</td>
<td>0.2</td>
<td>90.4</td>
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<td>Bathymodiolus manusensis</td>
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<tr>
<td>DG</td>
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<td>50.0</td>
<td>0.7</td>
<td>0.3</td>
<td>0.8</td>
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<td>0.1</td>
<td>0.5</td>
<td>1.0</td>
<td>0.1</td>
<td>0.1</td>
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<td>55.9</td>
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<td></td>
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<td>0.4</td>
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<td>DORM 2</td>
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<td>Tuna</td>
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<td>77.9</td>
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<td></td>
<td>3.9</td>
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</table>

*DG: Digestive Gland; D.L.: detection limit; n.c.: not certified

Table 4
Percentage of each arsenic species present in body parts from A. hessleri and I. nautilei and the mussel B. manusensis.

<table>
<thead>
<tr>
<th>Vent animal</th>
<th>organ</th>
<th>AsIII</th>
<th>AsV</th>
<th>DMA</th>
<th>SO4-sug</th>
<th>As-Bet</th>
<th>TMAP</th>
<th>Ac</th>
<th>TETRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alviniconcha hessleri</td>
<td>DG</td>
<td>81.7</td>
<td>15.7</td>
<td>0.3</td>
<td>0.8</td>
<td>42.2</td>
<td>14.7</td>
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<tr>
<td>Gill</td>
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<td>2.3</td>
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<tr>
<td>Muscle</td>
<td>48.0</td>
<td>9.8</td>
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<td></td>
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<tr>
<td>Average</td>
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<td></td>
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<td>Ifremeria nautilei</td>
<td>DG</td>
<td>82.3</td>
<td>10.2</td>
<td>0.9</td>
<td>6.0</td>
<td>0.9</td>
<td>0.7</td>
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<tr>
<td>Gill</td>
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<td>8.9</td>
<td>61.7</td>
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<tr>
<td>Muscle</td>
<td>64.7</td>
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<td></td>
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<tr>
<td>Bathymodiolus manusensis</td>
<td>DG</td>
<td>22.3</td>
<td>10.3</td>
<td>25.1</td>
<td>38.2</td>
<td>3.1</td>
<td>0.9</td>
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<tr>
<td>Gill</td>
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<td>5.0</td>
<td>20.9</td>
<td>45.9</td>
<td>2.3</td>
<td>4.5</td>
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<tr>
<td>Muscle</td>
<td>8.4</td>
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<td>91.6</td>
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<td>Average</td>
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</table>
similar to the gastropods in that there were no As-sugs. However, the mussel speciation was dominated in all body parts by As-Bet. Interestingly, the digestive gland and gills contained not only As\textsuperscript{III}, As\textsuperscript{V}, and As-Bet, but also high proportions of DMA\textsuperscript{V} (~20%), TMAP (2–3%), and TETRA (1–4.5%). TETRA was not detected in any of the other organisms, and may be a degradation product of As-Bet (Reimer et al., 2010). Similar to the gastropods, the muscle of this organism contained only As\textsuperscript{III} (8.4%), with the remaining arsenic entirely as As-Bet.

Comparing our data to those previously reported in the literature, Larsen et al. (1997) reported that As speciation of extracts of \textit{R. exoculata} tissues (tail muscles) showed a clear dominance of As-Bet (98–100%), with trace As\textsuperscript{III}, As\textsuperscript{V}, and DMA\textsuperscript{V}. No As-sugs were detected. \textit{B. puteoserpensis}, on the other hand, was dominated by As-sugs, specifically PO4- and GLY-sug (54–58 and 14–35%, respectively). As-Bet was the second most abundant (3.5–16.3%), whereas As\textsuperscript{III} (5.4–7.2%), As\textsuperscript{V} (0.5–0.75%), MA\textsuperscript{V} and DMA\textsuperscript{V} (trace) and one unknown arsenic species were also detected in this mussel. Taylor et al. (2012) indicated that the dominant As species in the shrimp \textit{R. exoculata} was As-Bet (64–81% of the water-extractable As), along with As\textsuperscript{III} (3–41%). Four unknown cationic species were also detected in the shrimp, but they were not identified, and only made up a small portion of the overall As species distribution. For the mussel \textit{B. azoricus} and the polychaete \textit{B. seepensis}, PO4-sug was most abundant (4–31% and 37–52%, respectively), followed by As\textsuperscript{III}, As-Bet, As\textsuperscript{V}, an unknown cationic species, and DMA\textsuperscript{V} (Taylor et al., 2012). Some samples of \textit{B. azoricus} also contained what was termed a Thio-As-sugPO4 (14–51%), and it was suggested this species was present in the other organisms but had degraded during the extraction procedure to PO4-sug (Taylor et al., 2012). Unfortunately, the gastropod \textit{P. smaragdina} was not analyzed for As species.

5.3. Extraction efficiencies limit our interpretations

Unfortunately, do to poor extraction efficiencies, we can only speculate as to how these organisms are synthesizing As-sug and As-Bet. For example, the amount of extracted arsenic for \textit{A. hessleri}, \textit{I. nautilei}, and \textit{B. manusensis} ranged from 30% to 58%, 17–29%, and 41–66%, respectively. Although total extraction of all species is the objective of any speciation analysis, this is frequently not accomplished (Francesconi, 2003; Francesconi and Kuehnelt, 2004), because it is difficult to find an extraction fluid in which species of very different polarities (e.g., water- vs. lipid-soluble species) are equally soluble. Additionally, very unpolars arsenicals are poorly extracted by methanol (Francesconi, 2003), so such As species (including lipid-bound As) may represent the “non-extractable As” in the studied tissues. With such low extraction efficiencies, any interpretation of biosynthesis pathways is limited. In the following text, we speculate on the biosynthesis pathways based on not only our data but also those reported for other deep-sea vent organisms to date. However, a robust interpretation of the actual biosynthesis pathways for As-sug and As-Bet cannot be made at this time. Future research on As biotransformation in deep-sea vent organisms should endeavor for more comprehensive extraction of organoarsenicals.

5.4. As cycling in hydrothermal vents and As bioaccumulation

The three studies of arsenic bioaccumulation and biotransformation in deep-sea vent organisms to date indicate that gastropods, mussels, and polychaetes can all contain some form of As-sug. Larsen et al. (1997) was the first to suggest that As-sug may be produced by the autotrophic bacteria associated with microbial mats and/or symbionts. However, as pointed out earlier, there is no evidence to date for the occurrence of As-sugs in deep-sea vent microbial mats (Taylor et al., 2012). Nonetheless, this single data point cannot preclude their occurrence. Like plants and photosynthesizing algae, a primary out-come of deep-sea vent microbial community metabolism is reduction of CO\textsubscript{2} and the production of sugars. The connection between As-sugs and photosynthesizing algae is not a coincidence. As a result of photosynthesis the sugars glucose, fructose and sucrose are produced. Plants use chlorophyll and light energy from the sun to combine carbon dioxide and water to produce sugar and oxygen. Instead of photosynthesis, this process in deep-sea vent ecosystems takes place as a function of chemosynthesis. Typically, the dominant electron donor in hydrothermal vent environments is H\textsubscript{2}S. Bacteria use the energy obtained from oxidizing H\textsubscript{2}S to reduce CO\textsubscript{2} to produce sugars, S\textsuperscript{0}, and ultimately SO\textsubscript{4}. This is likely the main reaction taking place in deep-sea hydrothermal vent microbial communities, as well as by many of the symbionts contained within the gills and tissues of deep-sea vent organisms. Once the sugar is produced, the formation of arsensugars can take place by the aforementioned pathways, specifically in two main steps: (1) transformation of inorganic AsV to DMA\textsuperscript{III} via the Challenger pathway, which utilizes S-adenosylmethionine synthase. This would also lead to the formation of TMAP and TETRA. (2) adenosyl nucleoside can be transferred from S-adenosylmethionine to DMA\textsuperscript{III}, followed by glycosidation to form arsensugars (Edmonds, 2000; Caumette et al., 2012).

6. Conclusions

Because hydrothermal fluids emanating at the seafloor often contain high concentrations of arsenic, they can be useful targets when evaluating the biological uptake, bioaccumulation and biotransformation of inorganic arsenic to organoarsenicals within marine organisms.

Unfortunately, until we are able to develop more universal methods for complete extraction of all organoarsenical species, we may only speculate as to the possible synthesis of As-sugs and As-Bet in deep-sea vent organisms. Direct synthesis of As-Bet, as explained, may take place by the Edmonds pathway. However, it is more difficult to explain the synthesis of As-sugs. The implications of the assumption that As-sugs can be produced via microbial chemooautrophotrophy (as well as phototrophy) are profound. If chemosynthesis can be linked directly to the production of As-sugs, then their occurrence can take place essentially anywhere where microbes can survive, and the prerequisite for As-sug production by \textit{photosynthesizing} organisms is not a requirement.

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