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Supplementary material

Arsenic speciation in food chains from mid-Atlantic hydrothermal vents

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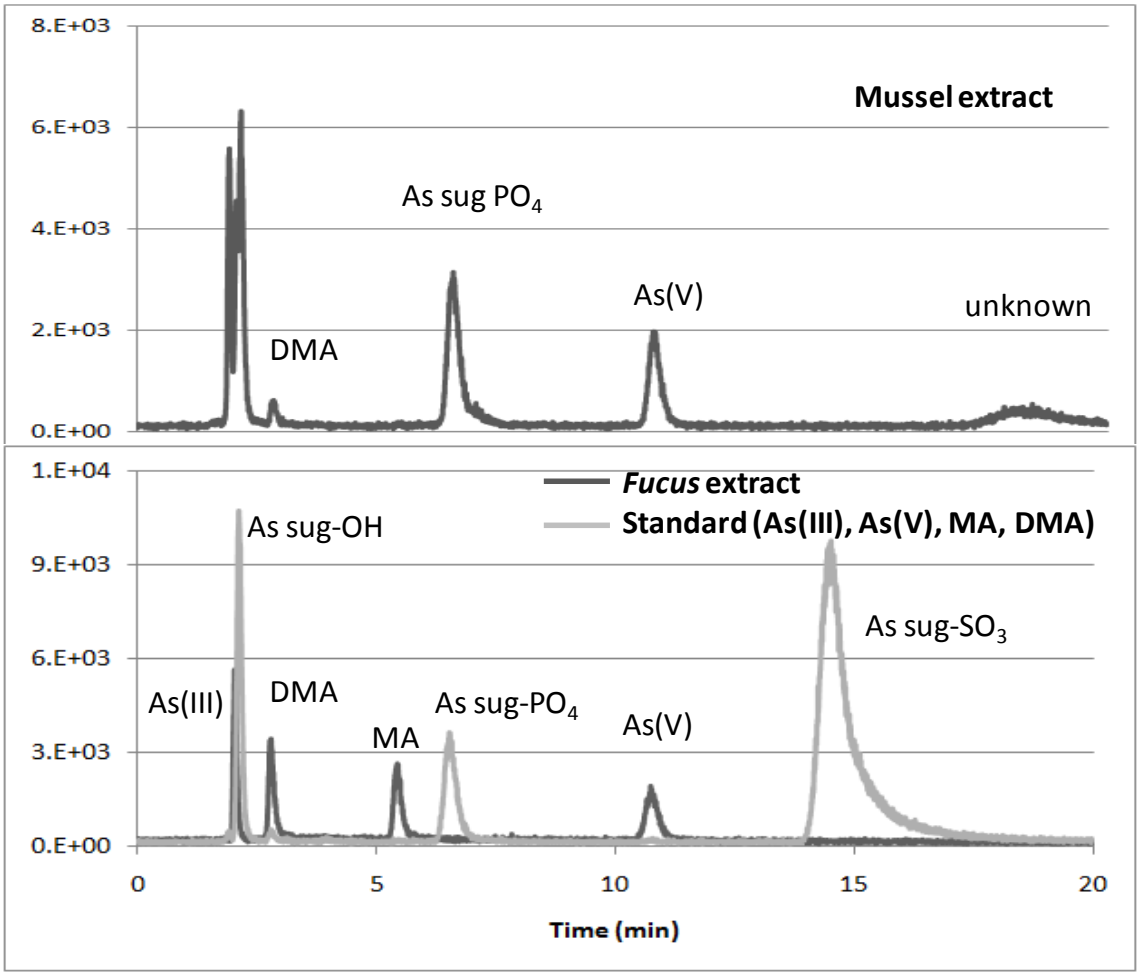

Fig. S1. Mussel (*B. azoricus*) extract (above) and standards and *Fucus* extract (below) analysed by anion exchange chromatography (PRP-X100 Hamilton column at 40°C with a mobile phase of 20 mM $NH_4H_2PO_4$ at pH 6). Column recoveries by this method were 91–96 % for shrimp (*R. exoculata*), 24–66 % for *B. azoricus* and 81–95 % for polychaetes (*B. seepensis*). The As-Sug-SO_4, also present in the *Fucus* extract, elutes at 31 min under these conditions.

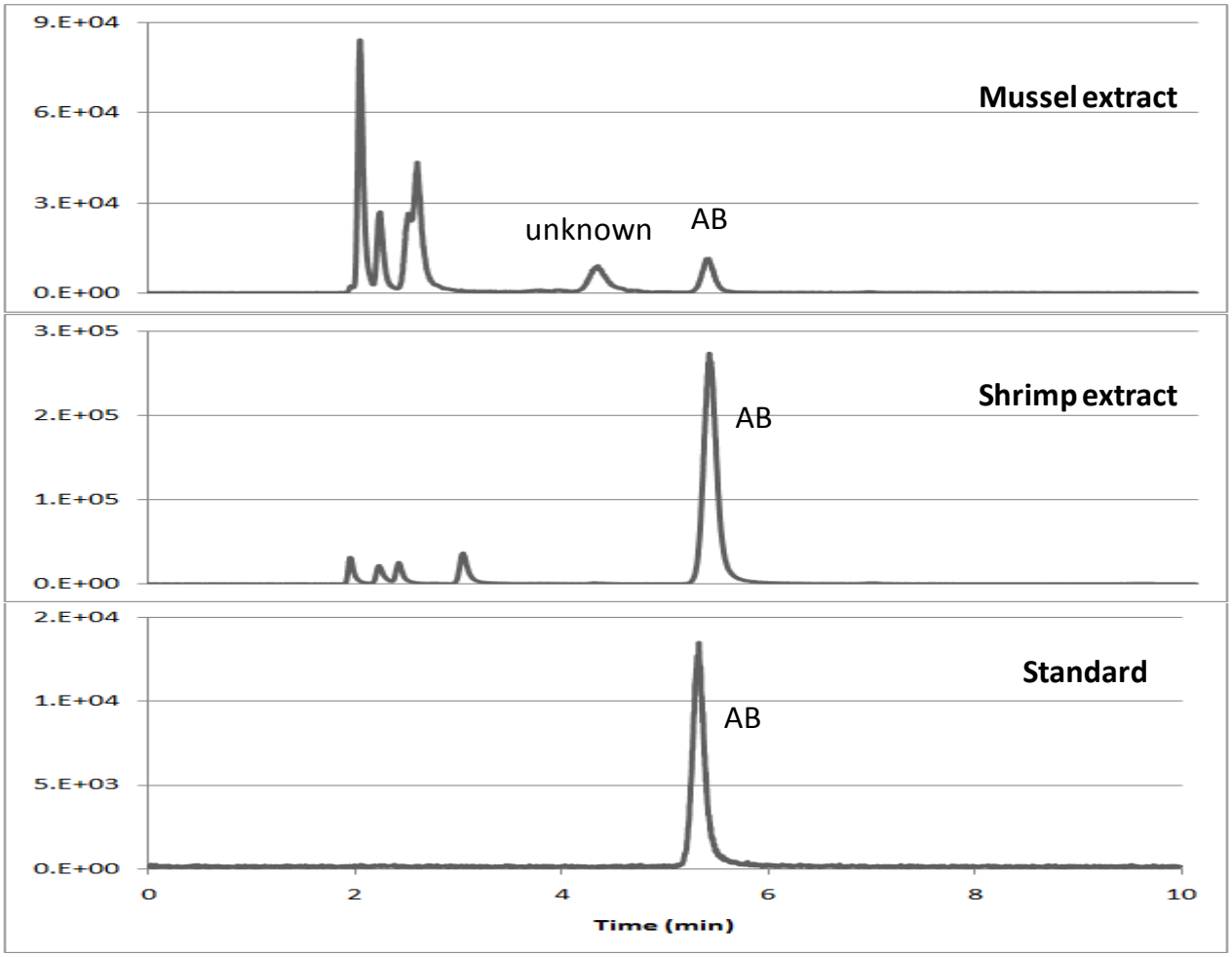

Fig. S2. Extracts of mussel (*B. azoricus*; top), shrimp (*R. exoculata*; middle) and AB standards (bottom) analysed by cation exchange chromatography (Supelcosil LC-SCX column with a mobile phase of 20-mM pyridine at pH 2). Column recoveries by this method were 98–103 % for shrimp, 14–52 % for mussel and 69–78 % for polychaetes (*B. seepensis*).

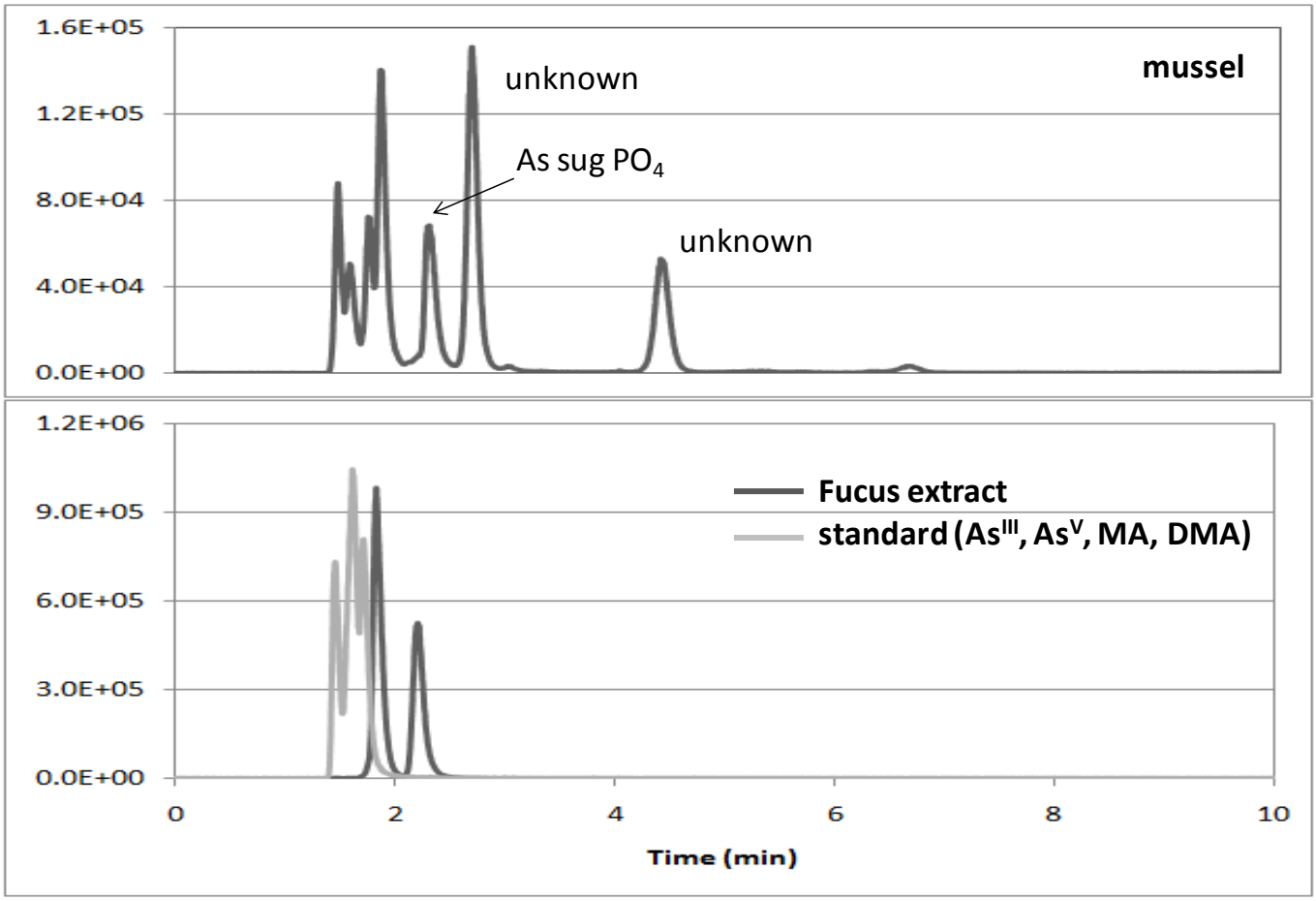

Fig. S3. A sample of *B. azoricus* (top), *Fucus* extracts and standard (bottom) analysed by reverse phase chromatography with a Shisheido Capcell PAK C18 MGII column and a mobile phase of 10-mM sodium 1-butansulfonate, 4-mM tetramethylammonium hydroxide, 4-mM malonic acid, 0.5 % methanol at pH 3. Column recoveries by this method were 58–80 % for *B. azoricus*. Only one peak (AB) was evident in the *R. exoculata* sample.

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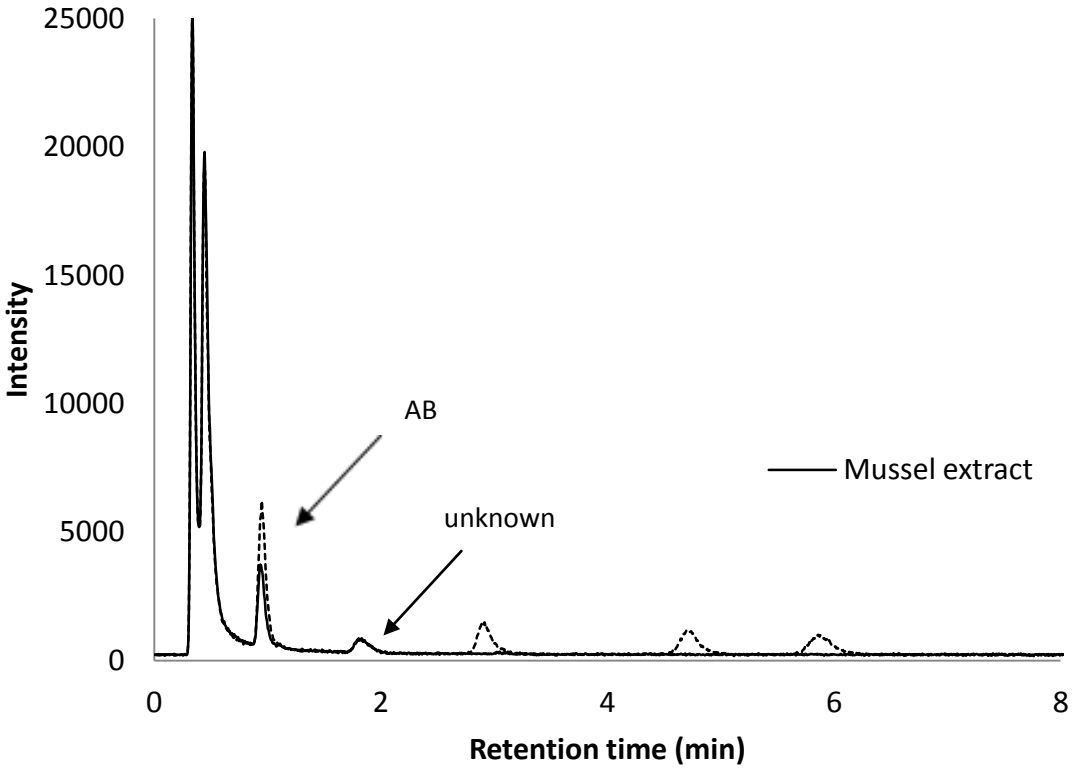

Fig. S4. Cation-exchange HPLC/ICPMS mass chromatogram of an extract of mussel, *B. azoricus* (solid line) and the same extract spiked with a standard solution containing AB, trimethylarsine oxide, arsenocholine and tetramethylarsonium ion (dotted line). Conditions were: Chrompack Ionospher 5C, with a mobile phase of 10-mM pyridine at pH 3. Column recovery was 96 %. The two peaks eluting at ~1 min. are a mixture of anionic species.

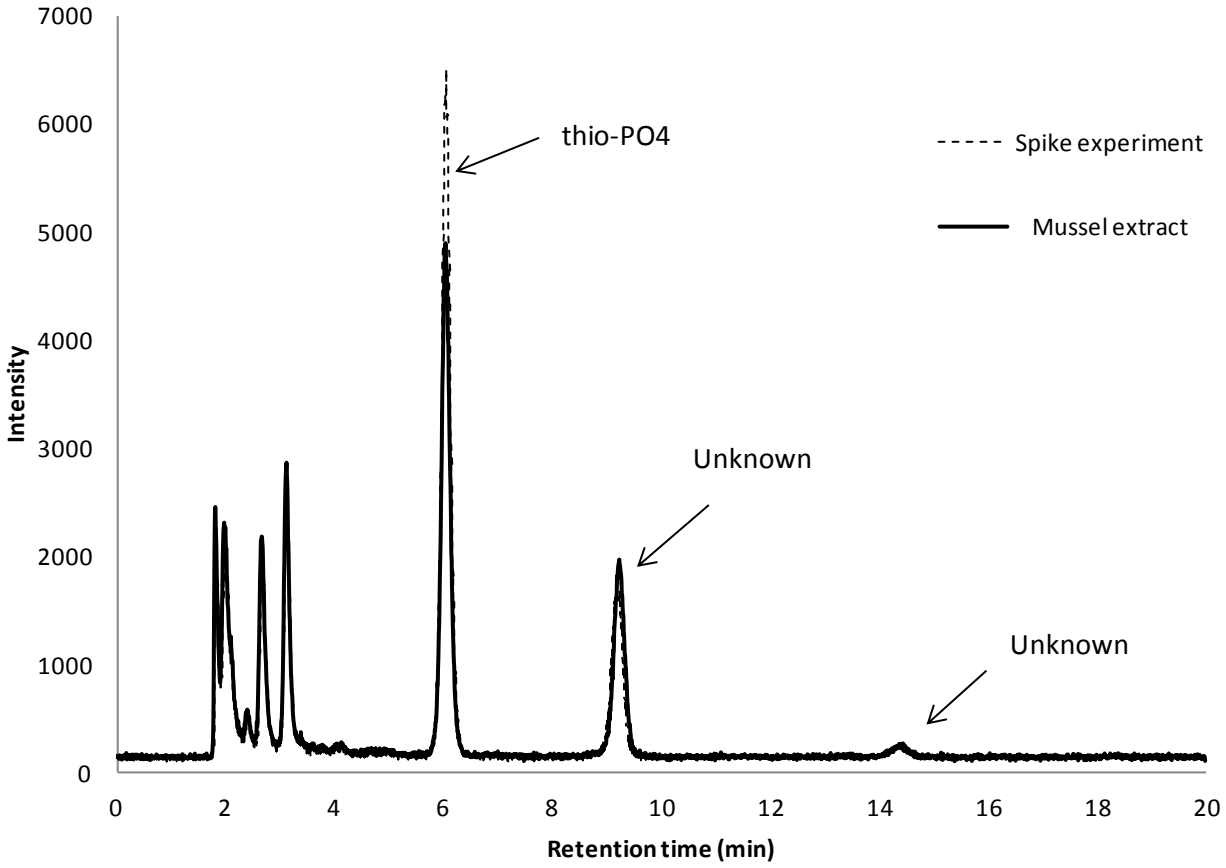

Fig. S5. Reversed-phase HPLC/ICPMS mass chromatogram of an extract of mussel, *B. azoricus* (solid line) and the same extract spiked with a standard solution of thio-arsenosugar PO_4 (dotted line). Conditions were Atlantis dC18 column with a mobile phase of 20 mM $NH_4H_2PO_4$ at pH 3. Column recovery was 81 %.

Table S1. Arsenic speciation in vent organisms from mid-Atlantic ridge

Site	Species	Body part	Total As	Extraction efficiency	Extracted As identified	AB	DMA	MA	As^{III}	As^{V}	Oxo-As $SugPO_4$	Thio-As $SugPO_4$	Unknown cation
			($mg\ kg^{-1}$)	(%)	(%)	($mg\ kg^{-1}$)							
Rainbow	*R. exoculata*	Tail	3.9	39	93	1.18	<0.004	<0.004	<0.002	0.22	<0.007	<0.007	<0.004
TAG	*R. exoculata*	Whole	29.8	64	105	12.3	<0.004	<0.004	<0.002	7.75	<0.007	N/A	<0.004
TAG	*R. exoculata*	Whole	34.5	65	105	15.4	<0.004	<0.004	<0.002	8.07	<0.007	N/A	<0.004
TAG	*R. exoculata*	Whole	25.0	71	89	14.3	<0.004	<0.004	<0.002	1.45	<0.007	<0.007	<0.004
Rainbow	*B. azoricus*	Whole	9.0	64	16	0.30	0.04	<0.004	0.09	0.23	0.23	N/A	0.18
Rainbow	*B. azoricus*	Whole	12.3	68	24	0.27	0.06	<0.004	0.68	0.51	0.53	N/A	0.23
Rainbow	*B. azoricus*	Whole	8.4	70	18	0.24	0.07	<0.004	0.16	0.29	0.31	N/A	0.59
Lucky Strike	*B. azoricus*	Whole	12.5	70	21	0.26	0.06	<0.004	0.46	0.27	0.80	N/A	0.33
Lucky Strike	*B. azoricus*	Whole	13.0	90	56	0.35	0.11	<0.004	1.12	2.04	2.98	N/A	1.15
Lucky Strike	*B. azoricus*	Whole	10.3	75	36	0.48	0.06	<0.004	0.46	0.60	1.15	1.1	0.48
Lucky Strike	*B. azoricus*	Whole	13.2	61	33	0.55	0.02	0.03	0.53	0.50	1.04	N/A	0.65
Lucky Strike	*B. azoricus*	Whole	6.0	69	64	0.30	0.04	<0.004	0.45	0.57	1.27	<0.007	0.49
Lucky Strike	*B. azoricus*	Whole	10.0	75	26	0.73	<0.004	<0.004	0.39	0.48	0.35	N/A	0.29
Lucky Strike	*B. azoricus*	Whole	11.4	76	19	0.40	0.04	<0.004	0.48	0.45	0.32	N/A	0.19
Lucky Strike	*B. azoricus*	Whole	16.0	49	23	0.39	0.04	0.07	0.47	0.61	0.25	N/A	0.13
Lucky Strike	*B. azoricus*	Whole	13.7	68	53	1.24	0.06	<0.004	0.67	1.11	1.90	N/A	0.59
Lucky Strike	*B. azoricus*	Whole	10.5	71	20	0.50	0.03	<0.004	0.50	0.22	0.22	2.3	0.17
Lucky Strike	*B. azoricus*	Whole	13.1	65	21	0.44	0.03	<0.004	0.61	0.38	0.35	2.4	0.39
Lucky Strike	*B. azoricus*	Whole	10.2	66	26	0.57	<0.004	<0.004	0.50	0.37	0.34	N/A	0.26
Lucky Strike	*B. seepensis*	Whole	20.4	85	77	1.40	0.63	0.01	2.81	2.17	9.10	N/A	2.48
Lucky Strike	*B. seepensis*	Whole	15.6	93	91	2.68	0.15	<0.004	1.66	2.44	6.25	N/A	1.36
Lucky Strike	*B. seepensis*	Whole	17.4	81	94	1.30	0.34	<0.004	2.69	1.71	7.28	<0.007	2.46
Lucky Strike	*B. seepensis*	Whole	14.3	95	79	2.33	0.37	<0.004	2.34	0.73	4.98	<0.007	2.18

Comparison of sample analysed at Dartmouth and Graz

Graz methods

A 40-mg sample was extracted by shaking with 2 mL of H_2O overnight. Aliquots (100 μL) of the extract were then subjected to microwave-assisted acid digestion before determination of total As concentration in the digest by ICPMS. Four analytical methods were used for As speciation – the anion exchange method (1) and oxidation with peroxide (3) were performed using the same procedures as at Dartmouth. Cation exchange chromatography was performed using a Chrompack Ionospher 5C (3 × 100 mm; Varian, Middelburg, the Netherlands) mobile phase 10-mM pyridine pH 3; flow rate 1.5 mL min^{-1}, injection volume 20 μL, and column temperature 40 °C. Reverse phase chromatography employed an Atlantis dC18 column (4.6 × 150 mm; Waters, Milford, MA, USA) with a mobile phase of 20 mM $NH_4H_2PO_4$ at pH 3 and a flow rate 1.5 mL min^{-1}, with an injection volume 20 μL and a column temperature of 30 °C.

Total As concentration was determined to be 10.3 and 8.3 mg kg^{-1} from the Dartmouth and Graz analyses. Higher extraction efficiency was achieved from the Dartmouth sub-sample (75 %) than the Graz sample (54 %). The difference in extraction may have been resulted from sample heterogeneity, or slightly more aggressive agitation of the sample (samples were sonicated for 1 h) at Dartmouth. Differences in concentrations of As species between the two studies were evident, but can be explained by the difference in extraction efficiency, as abundances of AB, DMA, and inorganic As relative to percentage extracted As were in good agreement (relative difference < 5 %). Both analytical protocols found arsenosugar-PO_4 to be the major species (representing 29 % (Dartmouth) and 35 % (Graz) of the extractable As), although the Graz study found a relatively greater portion of the arsenosugar to be in the thio-form than the oxo-form. This difference may be due to oxidation of the freeze-dried sample at Dartmouth during storage (the samples were stored at Dartmouth for several months longer than at Graz before analysis), or to oxidation of the sample extract before analysis. Both analytical schemes also separated an unknown species by cation exchange chromatography.

Table S2. Comparison of arsenic speciation (concentration of As species (percentage extracted As)) in a sample of *B. azoricus* analysed at Dartmouth and Graz

	Dartmouth (mg kg^{-1})	Graz (mg kg^{-1})
Thio-AsSug-PO_4	1.11 (14 %)	1.29 (29 %)
Oxo-AsSug-PO_4	1.15 (15 %)	0.29 (6 %)
AsB	0.48 (6 %)	0.31 (7 %)
As^{III} + As^{V}	1.06 (14 %)	0.57 (13 %)
DMA	0.06 (1 %)	0.05 (1 %)
As identified in extract	54 %	56 %
Extractable As	75 %	54 %
Total As	10.3	8.35