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Arsenic speciation in sea scallop gonads

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The arsenic species in male and female sea scallop gonads (*Placopecten magellanicus*) at pre-spawning and post-spawning stages, collected near Newfoundland, Canada, were characterized by using high-performance liquid chromatography–inductively coupled plasma-mass spectrometry. All samples contain arsenobetaine (0.5–3.0 μg g^{-1}) and a dimethylarsinoylriboside derivative as the major water-soluble arsenic compounds. Male pre-spawning gonads contain 0.35–2.4 μg g^{-1} of the dimethylarsinoylriboside. Female pre-spawning gonads contain 0.47–9.64 μg g^{-1} of the same compound. Post-spawning gonads, both male and female, contain higher concentrations of this compound (3.7–11.4 μg g^{-1}). The total concentrations of water-soluble arsenic compounds are different in male and female pre-spawning gonads. These differences are not obvious in post-spawning gonads. Post-spawning gonads contain higher total concentrations of water-soluble arsenic compounds than pre-spawning gonads. Copyright © 2001 John Wiley & Sons, Ltd.

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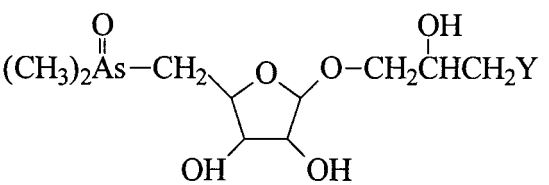
INTRODUCTION

Arsenic has been the focus of much recent research because of the toxicity of some chemical forms of this element. The concentration of arsenic compounds in marine organisms, many of which are food products for humans, is considerably higher than in seawater.[1]

None of the chemical forms of arsenic in marine organisms was known until 1977, when arsenobetaine was isolated from the western rock lobster in Australia.[2] Since then, a variety of arsenicals have been identified from marine animals and algae. For example, the tetramethylarsonium ion was found in clams,[3–5] arsenosugars (Table 1) were found in marine algae[6] and bivalves,[5] and arsenobetaine was found to be the major form of arsenic in marine animals.[6] These organoarsenic species are widely believed to be products of arsenate detoxification, although evidence for the high toxicity of methylarsenic(III) species is rapidly accumulating.[7–10] Until recently it was believed that only simple arsenic species, such as arsenite, arsenate, methylarsonic acid and dimethylarsinic acid, would be found in the terrestrial and freshwater environments; however, arsenobetaine and arsenocholine

Table 1 The major arsenosugar derivatives found in biological systems



1

Y

1a	-OH
1b	$-OPO_3HCH_2CH(OH)CH_2OH$
1c	$-SO_3H$
1d	$-OSO_3H$

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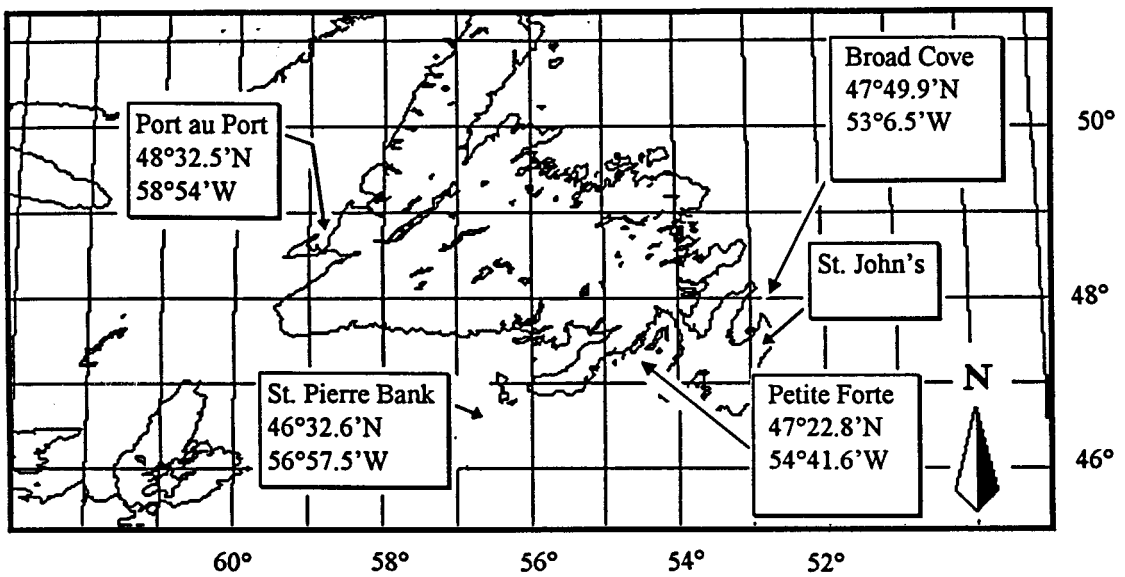

Figure 1 Map of Newfoundland showing the four sampling locations, Port au Port, St. Pierre Bank, Broad Cove and Petite Forte. These are the sites of commercial fisheries.

have been found in mushrooms,[11] and arsenosugars in a terrestrial alga *Nostoc sp.*,[12] and in earthworms.[13]

Previous studies have revealed that arsenic speciation in organisms varies with the seasons and the parts of the plant[14] and with the organs of scallops,[15] mussels,[16] and clams.[3–5] The present study shows that arsenic speciation in scallops may be dependent on the sex and the spawning cycle.

EXPERIMENTAL

Sample collection and preparation

Sea scallops (*Placopecten magellanicus*) were collected from four locations around Newfoundland (Port au Port (48 °32.5′N, 58 °54′W); St Pierre Bank (46 °32.6′N, 56 °57.5′W); Petite Forte (47 °22.8′N, 54 °41.6′W); Broad Cove (47 °49.9′N, 53 °6.5′W)), as shown in Fig. 1. Samples were frozen for transportation to the laboratory and stored at −20 °C until they were dissected. The gonads were isolated for the present study; tissues were then freeze-dried.

Reagents and chemicals

All chemicals used were of analytical grade unless otherwise stated and include: sodium arsenate heptahydrate ($Na_2HAsO_4{\cdot}7H_2O$, Sigma), arsenic (III) oxide (As_2O_3, Alfa products), monomethylarsonic acid ($CH_3AsO(OH)_2$, Pfalz & Bauer, Stamford), cacodylic acid ($(CH_3)_2AsO(OH)$, Aldrich), methanol (HPLC grade, Fisher), tetraethylammonium hydroxide (20 wt%, Aldrich), malonic acid (BDH) and nitric acid (69%, sub-boiling distilled, Seastar Chemicals). Arsenobetaine, was synthesized as described in the literature.[2] Deionized water with resistivity better than 18 MΩ was used for the extractions and to make up the eluent for high-performance liquid chromatography (HPLC).

The glassware and plasticware were cleaned by soaking in 2% Extran solution overnight, rinsing with water and deionized water, followed by a soak in 0.1 M HNO_3 solution overnight. They were then rinsed with deionized water and air-dried.

Analytical procedures

Freeze-dried gonad samples (0.25–0.5 g dry weight) were extracted by using a procedure similar to that described previously.[15] Each sample was weighed into a 15 or 50 ml centrifuge tube. To each tube was added 5 ml of a methanol/water mixture (1:1, v/v). The tube was sonicated for 10 min and centrifuged (bench top) for 10 min. After centrifugation, the supernatant was removed by means of a Pasteur pipette and placed in a round-bottomed

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Table 2 Operating parameters of ICP-MS

Forward r.f. power (W)	1350
Reflected power (W)	<10
Outer (cooling) gas flow rate ($l\,min^{-1}$)	13.8
Intermediate (auxiliary) gas flow rate ($l\,min^{-1}$)	0.65
Nebulizer gas flow rate ($l\,min^{-1}$)	1.002
Analysis mode	TRA, 1 s time slice
Quadrupole pressure (mbar)	9×10^{-7}
Expansion pressure (mbar)	2.4

flask. The extraction procedure was repeated an additional four times for each sample. The combined supernatants were evaporated to dryness and dissolved in 5 or 10 ml of deionized water prior to further analysis. Extracts were stored at −20 °C and transferred to the cold room (below 4 °C) on the day of analysis.

The HPLC system consisted of a Waters Model 510 delivery pump, a Reodyne Model 7010 injector valve with a 20 µl sample loop, and an appropriate column. The column used was a reverse-phase C_{18} column (GL Sciences Inertsil ODS, 250 mm × 4.6 mm, from Japan). A C_{18} guard column was used preceding the analytical column. The HPLC system was connected to the inductively coupled plasma (ICP) nebulizer via a PTFE tube (20 cm × 0.4 mm) and appropriate fittings.

A VG Plasma Quad 2 Turbo Plus ICP-mass spectrometer (MS; VG Elemental, Fisons Instrument), equipped with an SX 300 quadrupole mass analyzer, a standard ICP torch, and a de Galan V-groove nebulizer, was used as the detector. The time-resolved analysis (TRA) mode was used to set the mass analyzer to monitor the $m/z = 75$ signal peak corresponding to As^+. The TRA mode allowed simultaneous monitoring of more than one m/z value at a time. All signals were collected and the data were manipulated on a separate computer (MS Excel). A summary of the operating parameters for the ICP-MS is given in Table 2.

The mobile phase for the HPLC system consisted of 10 mM tetraethylammonium hydroxide (TEAH), 4.5 mM malonic acid, 0.1% MeOH, pH 6.8 (by HNO_3) and a flow rate of 0.8 ml/min^{-1} was used. All samples were filtered (0.45 µm) prior to injection onto the column. In general, 20 µl samples were analyzed. Arsenic compounds in the samples were identified by matching the retention times of the peaks in the chromatograms with those of standards and the standard reference material (SRM)

Quality assurance was maintained by analysis of a standard reference material SRM 1566a (oyster tissue) from the National Institute of Standards and Technology, US Department of Commerce. Kelp powder, a product from Eastern Canada, was purchased from a local food store in Vancouver, B.C., and used as laboratory reference material.

RESULTS

Identification of arsenic compounds

Arsenic compounds in the extracts were identified by matching the HPLC–ICP-MS retention times with those of peaks previously identified in chromatograms of extracts of oyster tissue NIST 1566a,[5] an in-house kelp powder standard,[12] and some available synthetic standards. The chromatograms were very similar to those obtained from other scallop samples.[15] Table 3 shows a summary of the speciation results of samples from four locations. It should be noted that the concentrations of arsenic species reported in Table 3 are based on calibration curves prepared from known standards

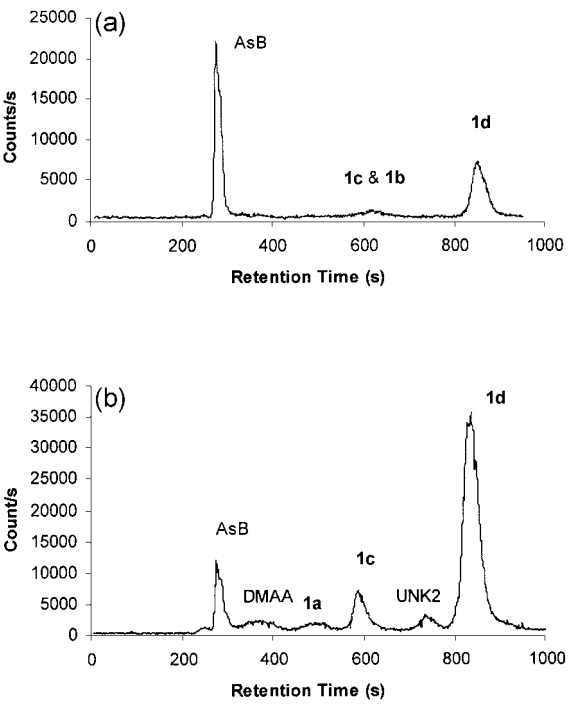

Figure 2 Chromatograms of male sea scallop gonad extracts from Petite Forte (2a: pre spawning; 2b: post spawning).

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Table 3 Arsenic speciation in scallop gonads ($\mu g_{As}\, g^{-1}$); Pre Sp: pre-spawning; Post Sp: post-spawning

Samples	Shell height (mm)	AsB	UNK1	DMAA	**1a**	**1c**	**1b**	UNK2	**1d**	Total
Sea scallops from Broad Cove										
Pre Sp., Male	129	1.05		0.12	0.09		0.19	0.12	0.74	2.30
Pre Sp., Female	149	1.93			0.18		0.47		1.32	3.90
Pre Sp., Female	127	1.80			0.12		0.52		0.47	2.90
Pre Sp., Female	113	2.08	0.74		0.19		0.63		1.35	5.00
Post Sp., Male	131	1.25	0.95		0.52		0.56	0.49	3.94	7.71
Post Sp., Male	125	0.69	0.54		0.35		0.68	0.36	3.68	6.30
Post Sp., Female	130	0.66	0.36		0.21		0.61	0.33	5.25	7.41
Post Sp., Female	131	2.79	0.67		0.57		1.57	0.41	7.19	13.20
Sea scallops from Petite Forte										
Pre Sp., Male	133	1.75				0.09	0.15	0.02	1.18	3.20
Pre Sp., Male	147	1.48				0.02	0.04	0.01	1.05	2.60
Pre Sp., Female	139	2.83		0.25	0.35	0.17	0.63	0.12	3.95	8.30
Pre Sp., Female	121	3.00			0.29	0.08	0.37	0.07	4.19	8.00
Post Sp., Male	125	1.14		0.65	0.46	0.86	0.40	0.56	7.35	11.41
Post Sp.,? Sex	125	1.42			0.44	0.97	0.67	0.59	11.53	15.62
Post Sp.,? Sex	144	1.02				0.40	0.32	0.42	6.44	8.60
Post Sp.,? Sex	143	1.33		1.08		1.83	0.69	1.47	16.51	22.90
Sea scallops from Port au Port										
Pre Sp., Male	86	0.63			0.04		0.10	0.05	1.47	2.30
Pre Sp., Male	89	0.98			0.07		0.15	0.05	2.45	3.70
Pre Sp., Male	98	0.49			0.00		0.12		0.49	1.10
Pre Sp., Female	88	1.56			0.34		0.74	0.08	2.77	5.50
Pre Sp., Female	102	1.11		0.31	0.49		0.96	0.29	9.64	12.80
Pre Sp., Female	92	1.31		0.37	0.40		0.66	0.26	8.31	11.30
Post Sp., Male	100	0.96			0.42	0.89	0.57	0.66	11.10	14.60
Post Sp., Male	95	1.28					0.24	0.39	7.50	9.40
Post Sp., Female	103	2.05			0.46	0.60	0.21	0.15	8.24	11.70
Post Sp., Female	110	1.32			0.33	0.69	0.26	0.40	11.40	14.41
Sea scallops from St Pierre Bank										
Pre Sp., Male	106	1.21			0.06		0.14	0.07	0.73	2.20
Pre Sp., Male	107	1.07					0.08		0.35	1.50
Pre Sp., Male	131	0.97		0.11	0.11		0.23	0.14	1.33	2.90
Pre Sp., Female	130	0.96		0.28	0.31		0.57	0.22	3.07	5.40
Pre Sp., Female	129	0.87			0.36		0.50	0.46	5.22	7.40
Pre Sp., Female	110	0.86			0.19		0.37	0.16	2.31	3.90
Pre Sp., Female	126	0.51			0.19		0.36	0.15	2.59	3.80

when possible. Calibration curves from dimethylarsinic acid were used to quantify arsenosugars and unknowns. Typical chromatograms are shown in Figure 2.

Petite Forte (Fig. 1)

Samples collected from this site consist of male and female pre-spawning gonads, one male post-spawning and three post-spawning gonads of undetermined sex. The shell heights of the scallops range from 121 to 147 mm.

All male pre-spawning gonads and all post-spawning gonads contain similar concentrations of arsenobetaine (1.0–1.75 $\mu g\, g^{-1}$). Female pre-spawning gonads contain higher concentrations of arsenobetaine (2.8–3.0 $\mu g\, g^{-1}$). The concentration of arsenosugars (especially **1d**, Table 1) is higher in female pre-spawning gonads (3.95–4.19 $\mu g\, g^{-1}$) than that in male pre-spawning gonads (1.1 $\mu g\, g^{-1}$). All post-spawning gonads contain higher concentrations of arsenosugar **1d** and total amounts of arsenic compounds than pre-spawning gonads.

Broad Cove (Fig. 1)

Samples collected from this location consist of male and female, pre- and post-spawning gonads

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Table 4 Summary of gonad speciation data for selected arsenic species (all four locations; $\mu g\ g^{-1}$)

Samples	Arsenobetaine	**1d**	Total (sum of peaks)
Male-pre-spawning		0.35–2.4	1.5–3.7
Female pre-spawning	0.5–3.0	0.47–9.64	2.9–12.8
Male post-spawning		3.68–11.1	6.3–14.6
Female post-spawning		5.25–11.4	7.4–14.4

with shell heights from 113 to 149 mm. The concentration of arsenobetaine in female pre-spawning gonads (1.8–2.1 $\mu g\ g^{-1}$) is higher than that in male pre-spawning gonads (1.05 $\mu g\ g^{-1}$). Arsenobetaine in post-spawning gonads varies from 0.6 to 2.8 $\mu g\ g^{-1}$. It should be noted that an unknown compound, UNK1, Table 3, was found in all post-spawning gonads from this site and in only one female pre-spawning gonads. UNK1 was not detected in gonad extracts from any other site. UNK2 was found at all sites at very similar concentrations to **1a**, Table 3. The total concentrations of arsenic species, mainly arsenosugars, in female pre-spawning gonads (total: 2.9–5 $\mu g\ g^{-1}$) are slightly higher than those in the male pre-spawning gonads (2.3 $\mu g\ g^{-1}$).

Female post-spawning gonads contain slightly higher concentrations of arsenosugar **1d**, Table 3, and total arsenic than male post-spawning gonads. The concentrations of **1d** and total arsenic in pre- and post-spawning gonads are significantly different.

Port au Port (Fig. 1)

Samples collected from this site consist of gonads of both sexes and at both stages of spawning. Their shell heights are 86 to 110 mm. The trend in concentration of arsenobetaine in samples collected from this site is similar to that of samples collected from the two sites mentioned above. Female pre-spawning gonads contain slightly higher concentrations of arsenobetaine (1.1–1.56 $\mu g\ g^{-1}$) than do male pre-spawning gonads (0.49–0.98 $\mu g\ g^{-1}$). Post-spawning gonads contain higher concentrations of arsenobetaine (0.9–2.0 $\mu g\ g^{-1}$) than pre-spawning gonads (0.49–1.56 $\mu g\ g^{-1}$). The concentrations of **1d** (2.77–9.6 $\mu g\ g^{-1}$) and total water-soluble arsenic species (5–12.8 $\mu g\ g^{-1}$) in female pre-spawning gonads are higher than those of males (0.4–2.4 $\mu g\ g^{-1}$ of **1d**, 1.1–3.7 $\mu g\ g^{-1}$ of total). These differences between sexes were not observable in post-spawning gonads. Post-spawning gonads of both sexes contain higher concentrations of **1d** and total arsenic than pre-spawning gonads.

St Pierre Bank (Fig. 1)

Samples collected from this site consist of pre-spawning gonads only. The shell heights are 106 to 131 mm. The concentrations of arsenobetaine in gonads of different sex are similar. Female gonads contain higher amounts of **1d** (2.3–5.2 $\mu g\ g^{-1}$) and total arsenic (3.8–7.4 $\mu g\ g^{-1}$) than do male gonads (0.35–1.3 $\mu g\ g^{-1}$ of **1d** and 1.5–2.9 $\mu g\ g^{-1}$ of total arsenic).

DISCUSSION

A summary of some data from the present study is given in Table 4. There is no obvious relationship between the shell heights and the total water-soluble arsenic concentrations in the scallops. The concentration of arsenobetaine in all samples lies in the narrow range of 0.5 to 3 $\mu g\ g^{-1}$. The concentration of **1d** and the sum of arsenic species in male and female pre-spawning gonads are different. It is notable that these sex-dependent differences are less obvious in post-spawning gonads.

Little has been published on arsenic speciation of scallops.[15] Most previous studies reported only on the arsenic concentration in the muscle of scallops.[1,17,18] In the present study, we have focused on the arsenic speciation in the gonads of scallops. Arsenosugars, which are believed to be non-toxic to humans, are found to be the major water-soluble arsenic species in gonad extracts. However, there are significant differences in the concentrations of arsenosugar **1d** in female and male gonads at the pre-spawning stage, a finding that may be related to the reproductive cycle. It is perhaps less remarkable to find that the sum of the concentrations of water-soluble arsenic species detected in the post-spawning gonads is higher than in the pre-spawning gonads. This 'increase' in the concentration may

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simply be related to the decrease in gonad mass that accompanies spawning; however, the change in species distribution may also be related to the spawning.

These findings raise numerous questions about the role of arsenic compounds in nature and about the mechanisms and pathways of conversion of arsenic species. We need to know which arsenic species are retained by the gonads and which are transferred to the next generation, and why. We also need to know if the concentrations of arsenic species are related to their chemical properties, or to the physical properties of different tissues in the organisms. We are attempting to answer these questions in more detail by monitoring the arsenic speciation of scallops at different stages of their life cycles. Some preliminary data on the spiny scallop, *Chlamys hastata*, and the hybrid Pacific scallop support the conclusions reached above.[19]

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