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Seasonality in estuarine sources of methylated arsenic

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The effect of seasonal temperature change on the release of methylated arsenic from macroalgae, phytoplankton and sediment porewaters has been investigated by a series of controlled laboratory experiments. The appearance of dissolved arsenic species in the overlying waters was monitored using a coupled hydride generation/GC AA analytical technique. The liberation of dissolved arsenic species by the macroalgae *Ascophyllum nodosum* was examined under estuarine conditions at 5 °C and 15 °C. At the lower temperature the release rates were 0.2 μg kg^{-1} h^{-1} (wet weight of material) for monomethylarsenic (MMA) and 0.5 μg kg^{-1} h^{-1} for dimethylarsenic (DMA), whereas at 15 °C the rates were 0.4 μg kg^{-1} h^{-1} and 3.2 μg kg^{-1} h^{-1}, respectively. Incubation experiments were also carried out at 15 °C using the diatom *Skeletonema costatum*. During the log growth phase, when chlorophyll *a* concentrations were in the range 1–5 μg dm^{-3}, the rate of appearance of DMA in the water was ~3 ng dm^{-3} h^{-1}. Sediment samples from the freshwater and seawater end-members of the Tamar Estuary, UK, were incubated under natural conditions at 5 °C and 15 °C. The freshwater sediments released DMA in preference to MMA; the concentrations of both species increased exponentially and reached a steady state in the overlying water after 250 h. Considerably more DMA was produced at 15 °C than at 5 °C, whilst the amount of MMA produced appeared to be insensitive to the temperature increase. In contrast, the seawater sediments always produced more MMA than DMA and the increase in temperature had little effect on the production of either MMA or DMA.

The results of the laboratory experiments were compared with field observations in temperate estuaries, including the Tamar Estuary. The implications of changes of water temperature on the fate of arsenic in estuaries is discussed and modifications to the estuarine arsenic cycle are proposed.

Keywords: Arsenic, methylation, macroalgae, phytoplankton, natural water, seasonal variation

INTRODUCTION

Biological activity can mediate the environmental chemistry of many trace metals,[1] including arsenic, in an element whose biogeochemistry plays a crucial role in its mobility in natural waters.[2–4] Marine biomethylation of arsenic has been associated with phytoplankton because of their need to take up phosphate from the water column. Difficulties arise during this process because arsenate is chemically similar to phosphate and the phosphate/arsenate concentrations may be close to equimolar in the coastal boundary zone.[5] Phytoplankton discrimination between arsenate and phosphate at equimolar concentrations is relatively poor, being only a factor between two and ten.[6] In biologically productive waters, phytoplankton may take up arsenate, leading to poisoning by uncoupling of the oxidative phosphorylation mechanism. Phytoplankton cells metabolize arsenate to methylated species and more complex molecules, such as arseno-sugars.[3] The final consequence of these biochemical processes is that dissolved methylated arsenic species have been detected in estuaries and coastal waters. Because analytical methods normally only determine the degree of methylation, the terms monomethylarsenic (MMA) and dimethylarsenic (DMA) will be used in this paper. Some attention has been paid to the sources of these compounds, although there are still considerable gaps in our understanding of arsenic biogeochemistry in estuaries.[3]

Surveys of arsenic species in productive estuaries of the UK[7–11] and the USA[12,13] and in shelf seas[14–17] have shown that the detection of MMA and DMA can be dependent on a variety of factors, including salinity, temperatures, nutrient

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availability and plankton species. The concentrations of DMA usually exceed those of MMA, although in some cases[7–9] the concentrations of MMA are close to or exceed those of DMA. This differential behaviour of DMA and MMA is related to estuarine biogeochemical processes, many of which are poorly understood. Furthermore, whilst MMA and DMA are the most commonly detected species in marine waters, Howard and Comber[18] have shown that other, more complex, organoarsenic compounds could be present.

Estuaries in the UK show a seasonality in the appearance of methylated species, such that they are detected when the water temperature exceeds 12 °C. The appearance of methylated species in these estuaries has been linked with the presence of phytoplankton and has been variously ascribed to their exudates or to the results of bacterial decay of their tissue.[19] However, there are other sources of methylated species. Macroalgae can contain significant concentrations of DMA, while MMA concentrations are negligible.[20,21] An additional source of methylated arsenic species could be from infusions of sediment porewaters.[22,23] Methylated arsenic species were detected in the porewaters of the Tamar Estuary[22] but the annual average concentrations of MMA was greater than DMA In the porewaters of marine sediments, whereas the reverse was the case in porewaters of sediments from the freshwater zone.

However, the relative importance and the seasonal interplay between each of the sources are not known; this is particularly the case for the Tamar Estuary, which is an active source of arsenic from previous mining activity.[24] The objective of this study was to examine the effects of temperature change under carefully controlled laboratory conditions, using natural material from the Tamar Estuary. Experiments of this kind which yield mechanistic and kinetic information are valuable in the further development of quantitative biogeochemical models[25] which can be used in the accurate prediction of the fate of contaminants following natural or man-made events.

METHODS

Sample collection and analysis

Sediments

Surface sediment samples were collected (in 1986) using a PTFE spatula at low tide, and they were stored in acid-washed polythene containers. The samples were returned to the laboratory, where they were immediately washed with Milli-Q water to remove seawater salts and freeze-dried for 48 h. Total metal analyses were carried out on about 2 g of sediment using aqua regia held at 110 °C for 4 h in a PTFE digestion bomb.[8] Available arsenic was determined using 5–10 g of sediment which was leached with 25% acetic acid at room temperature for 12 h in a sealed glass tube. The sediment extracts were analysed directly using graphite furnace atomic absorption (GF AA).[8]

Macroalgae

Samples of macroalgae were collected (in 1986) at low tide, washed several times in their native estuarine water to remove any debris and stored in sealed polythene containers for transportation to the laboratory. Algal samples were digested by refluxing, with concentrated hydrochloric acid, an air-dried (ground with a mortar and pestle) subsample (0.5–1.0 g) at 70 °C for 24 h.[21] The resultant leachate was filtered via a glass fibre filter into a volumetric flask, which was made up to the mark with deionized, doubly distilled water. The arsenic species were determined using the coupled hydride generation/GC AA system described below.

Analytical methods for arsenic species

Natural water samples and macroalgal extracts were analysed for inorganic and organic arsenic compounds by means of a coupled liquid-nitrogen trap, hydride generation/GC AA system. Gaseous covalent hydrides were generated from sodium arsenate, monomethylarsinic acid (disodium salt) and dimethylarsinic acid (sodium salt); the hydrides were collected in a liquid-nitrogen-cooled trap, which was then connected to a gas chromatograph via a switching valve and heated, causing the hydrides to be evolved. The separated hydrides were detected by GC AA, as previously described.[8,21,22] The detection limits were 0.02 $\mu g\,dm^{-3}$ for inorganic arsenic, 0.01 $\mu g\,dm^{-3}$ for MMA and 0.02 $\mu g\,dm^{-3}$ for DMA.

Laboratory simulations

Experiments with Macroalgae

The release of arsenic species from the Tamar Estuary macroalga *Ascophyllum nodosum* was

examined at 5 °C (representing winter conditions) and 15 °C (representing summer conditions). Approximately 180 g (wet weight) of fresh macroalgae was placed in 10 litres (dm^3) of filtered seawater (passing a 0.45 μm poresize filter), which was gently aerated for seven days. Nutrient broth was not added and the experimental chambers were kept in constant-temperature rooms. The experiments involved incubation of the samples in 'normal' light, with a quantum flux of 3.05×10^{15} quanta s^{-1} cm^{-2} in the 400–700 nm range, with a photo-period of 16 h light and 8 h dark. Water samples were taken at strategic times for the identification and quantification of arsenic species by means of coupled hydride generation/GC AA.

Experiments with diatoms

The diatom *Skeltonema costatum* was used as the test organism and axenic stock was obtained from batch cultures grown at the Marine Biological Association, Plymouth. These were cultured in filtered seawater (passing a 0.45 μM filter) which had been sterilized by autoclaving. Nutrients, 20 μM each of nitrate and silicate, were added to promote cellular growth. The inoculum of cells from the stock culture was designed to give a cell density of about 10^6 cells per dm^3. the *Skeletonema costatum* were placed in a 10-dm^3 glass container and kept in the 15 °C constant-temperature room under the light conditions described above. Sodium arsenate was added aseptically to the culture to give a concentration of 10 μg dm^{-3}. The experiment was run for several days until the culture had reached a stationary phase. An abiotic control experiment, containing autoclaved seawater and sodium arsenate but no cells, was run concurrently with the biotic one. The autoclaved reagents were tested for sterility by streaking on nutrient agar and soil-extract samples. Sub-samples were removed at strategic intervals for analysis for cell counts, chlorophyll *a* (by *in vivo* fluorescence measurements), reactive phosphate and concentrations of arsenic species.

Experiments with sediment porewaters

The experiments encompassed the end-members of the estuarine regime, i.e. water and sediment samples from the upper Tamar Estuary which were in contact with freshwater at all times, and water and sediment samples from a seawater lake near the mouth of the estuary. In both cases Tamar Estuary sediments (25 g wet weight) and 150 cm^3 of water (passing through a 0.45 μm poresize Millipore filter) were placed in 250 cm^3 glass reactors. Nutrient broth I (0.1%), D-glucose (0.03%) and yeast extract (0.03%) were added to promote microbial growth. The samples were kept under the same illumination conditions as above. An appropriate abiotic control was run concurrently, using sediment, water and nutrients sterilized by three separate autoclavings, each of 30 min. The autoclaved reagents were tested for sterility by streaking on nutrient agar and soil-extract agar samples. In all experiments the reactors were capped with an aseptic air filter, and freshwater, seawater and control reactors were allowed to equilibrate with the atmosphere in constant-temperature rooms maintained at 5 °C and 15 °C. Filtered aqueous sub-samples, removed from each reactor at strategic intervals over 1000 h, were analysed for arsenic species by means of coupled hydride generation/GC AA.

RESULTS AND DISCUSSION

Macroalgae

The concentrations of arsenic species in Tamar Estuary macroalgae collected in March 1986 are given in Table 1. The range for DMA was 3.4 to 33.8 μg g^{-1}, with the reproductive organs (sporangia) having higher concentrations of DMA than the stem. However, there are no discernable trends in arsenic species concentration along the estuarine gradient. In addition, these March samples from St John's Lake have DMA concentrations some four to five times higher than those collected in December 1985. The augmentation of the DMA concentration in March suggests a probable relationship to the increase in biological activity which takes place in spring. This hypothesis is supported by studies carried out by Klumpp,[20] who showed that the uptake of inorganic arsenic by *Fucus spiralis* is approximately doubled when the water temperature is raised from 16 °C to 30 °C. Given that methylation of inorganic arsenic is a known natural process, it could be that the increased arsenic uptake leads to higher concentrations of DMA.

Samples of the seaweed collected in March 1986 from St John's Lake were incubated and the results for 5 °C and 15 °C are shown in Figs 1 and 2, respectively. The appearance of methylated arsenic in the water containing the macroalgae

Table 1 Concentrations of arsenic species in macroalgal specimens collected from the Tamar Estuary

Season	Site	Distance up-estuary (km)	Specimen	Plant section	Mean arsenic concentration[a] ($\mu g\ g^{-1}$, dry wt)		
					Inorganic As	MMA	DMA
March 1986	Halton Quay	23	*Fucus vesiculosus*	Stem	0.45	0.15	14.20
				Sporangia	0.85	0.44	33.80
March 1986	Cargreen	19	*Ascophyllum nodosum*	Stem	0.97	<0.02	14.60
				Sporangia	0.46	0.19	29.80
			Fucus serratus	Whole plant	0.94	0.07	26.90
March 1986	Riverside	14	*Ascophyllum nodosum*	Stem	0.06	0.09	6.10
				Sporangia	0.08	0.15	22.70
March 1986	St John's Lake	7	*Ascophyllum nodosum*	Stem	1.42	0.13	13.57
				Sporangia	0.71	0.32	28.40
December 1985	St John's Lake	7	*Ascophyllum nodosum*	Stem	0.14	<0.05	3.41
				Sporangia	0.09	<0.05	6.14

[a] Hydrochloric acid digestion at 70 °C for 24 h. The means are results of three replicate analyses. Inorganic As refers to total As(III) + As(V).

may occur as a result of bacterial oxidation of arseno-sugars present in the outer cellular membranes.[6] The results for 5 °C show inorganic arsenic slowly increasing from the outset of the experiment. However, DMA was only above the detection limit after a delay of about 20 h and MMA after about a 50 h delay. Clearly, even though the concentration of DMA In the tissue of Tamar Estuary macroalgae is significant (see Table 1), the microbial degradation processes needed to release it to the water column are not highly active at 5 °C. In contrast, at 15 °C the

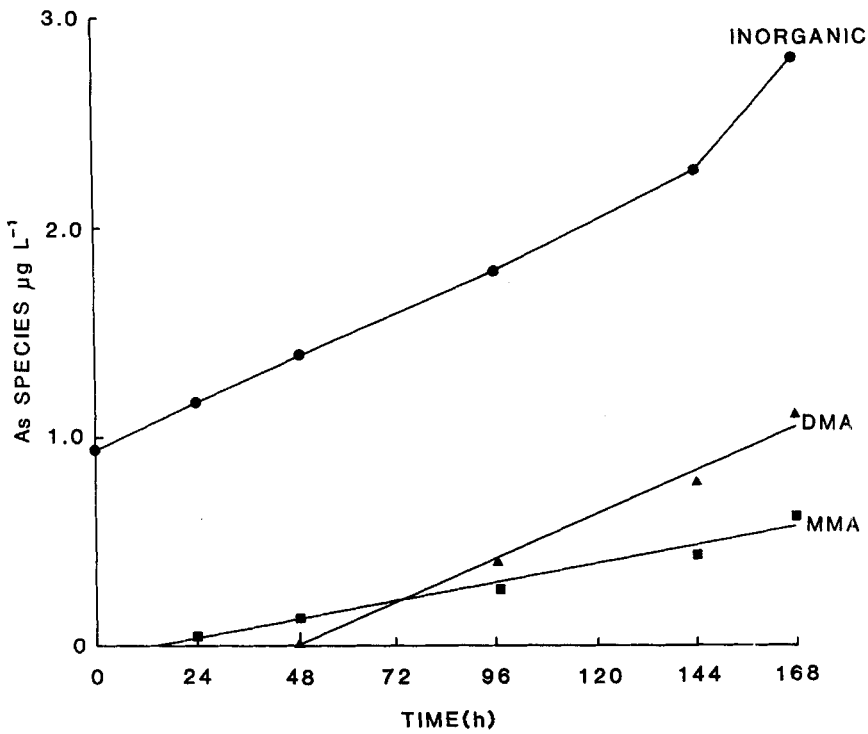

Figure 1 Concentrations of arsenic species ($\mu g\ dm^{-3}$) liberated as a function of time by *Ascophyllum nodosum* at 5 °C.

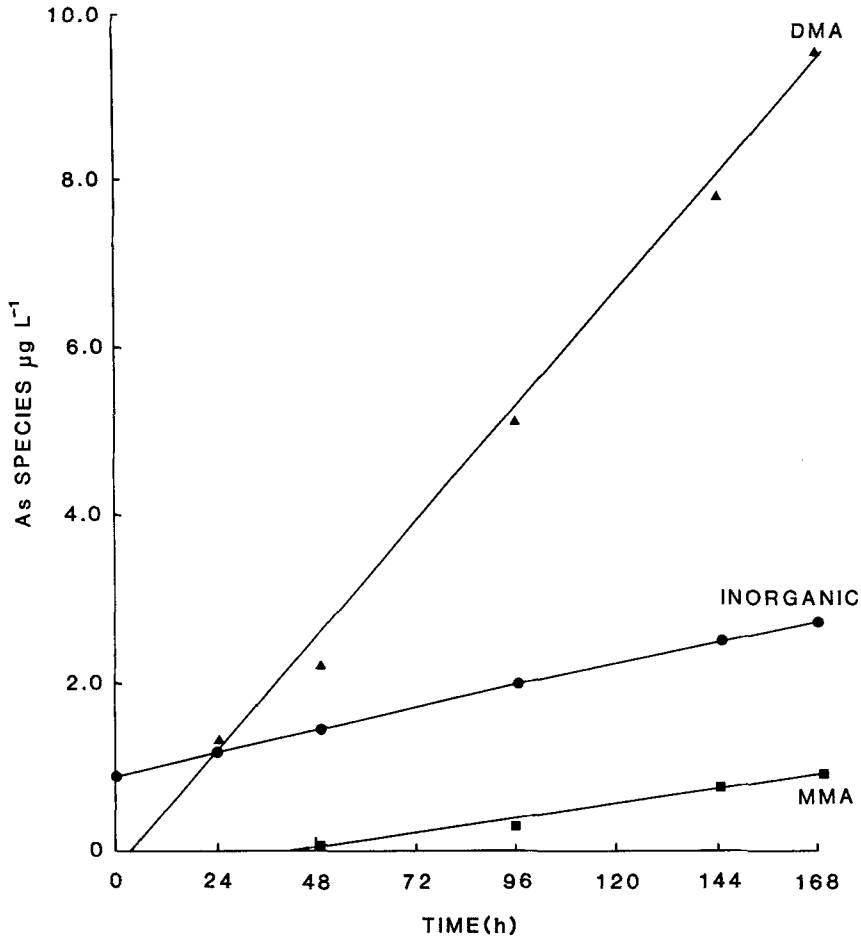

Figure 2 Concentrations of arsenic species ($\mu g\ dm^{-3}$) liberated as a function of time by *Ascophyllum nodosum* at 15 °C.

release of inorganic arsenic and DMA began immediately with a zero-order rate, but MMA was below the detection limit of the analytical technique until about 50 h after the start of the experiment, presumably because of the relatively low concentrations of MMA in the seaweed tissues (see Table 1). The rate at which inorganic arsenic was released into the water column as the temperature increased was not significant, but the rate of release of DMA was found to be a temperature-dependent process. Higher concentrations of DMA were released into the water at 15 °C compared with 5 °C, probably because of greater bacterial oxidation of algal cells.[3] At the higher temperature, the zero-order rate of appearance of DMA, deduced from the gradient in Fig. 2, is $3.2\ \mu g\ kg^{-1}\ h^{-1}$ and for MMA it is $0.4\ \mu g\ kg^{-1}\ h^{-1}$, based on the wet weight of the macroalgae. From Fig. 1 the release rate (after the delay period) at 5 °C for MMA is $0.2\ \mu g\ kg^{-1}\ h^{-1}$ and for DMA it is $0.5\ \mu g\ kg^{-1}\ h^{-1}$. Data on the rate of release of methylated arsenic species are not available, but Klumpp[20] has determined the uptake of inorganic arsenic by the same species of macroalgae to be $2.1\ \mu g\ kg^{-1}\ h^{-1}$ under conditions (temperature 13 °C; photo-period 12 h) comparable with the higher temperature. This uptake rate is similar to the rate of release for DMA reported here, suggesting that, under steady-state conditions and in warm waters, once inorganic arsenic has been taken up by algae it is rapidly methylated and released into the water column. The presence of large macroalgae colonies in the Tamar Estuary may therefore significantly influence the cycling of arsenic, a situation which possibly applies in other biologically productive estuaries such as the Beaulieu[7] and Southampton Water.[10]

Phytoplankton

The results from the incubation of the diatoms *Skeletonema costatum* in seawater at a temperature of 15 °C are shown in Figs 3 and 4. As reactive phosphate was taken up the cell density increased exponentially from 1×10^6 cells dm^{-3} at the start to a stationary-phase concentration of 35×10^6 cells dm^{-3} after seven days of incubation (see Fig. 3). The growth in cell density was linked with an increase of chlorophyll *a* concentrations from 1 to 5 μg dm^{-3}, which is typical of concentrations found in the lower Tamar in summer.[9] A small decrease in total dissolved inorganic arsenic (from 10.4 to 9.9 μg dm^{-3}) was observed, together with an exponential increase in the concentration of DMA (from <0.02 to 0.47 μg dm^{-3}), as shown in Fig. 4. The concentration of MMA was below its detection limit throughout the experiment. An abiotic control experiment (results not shown) gave negligible changes in arsenic and phosphate content, and DMA was always below the detection limit.

The release of DMA into the water coincided with logarithmic cell growth (one to seven days). During the stationary cell growth phase, DMA

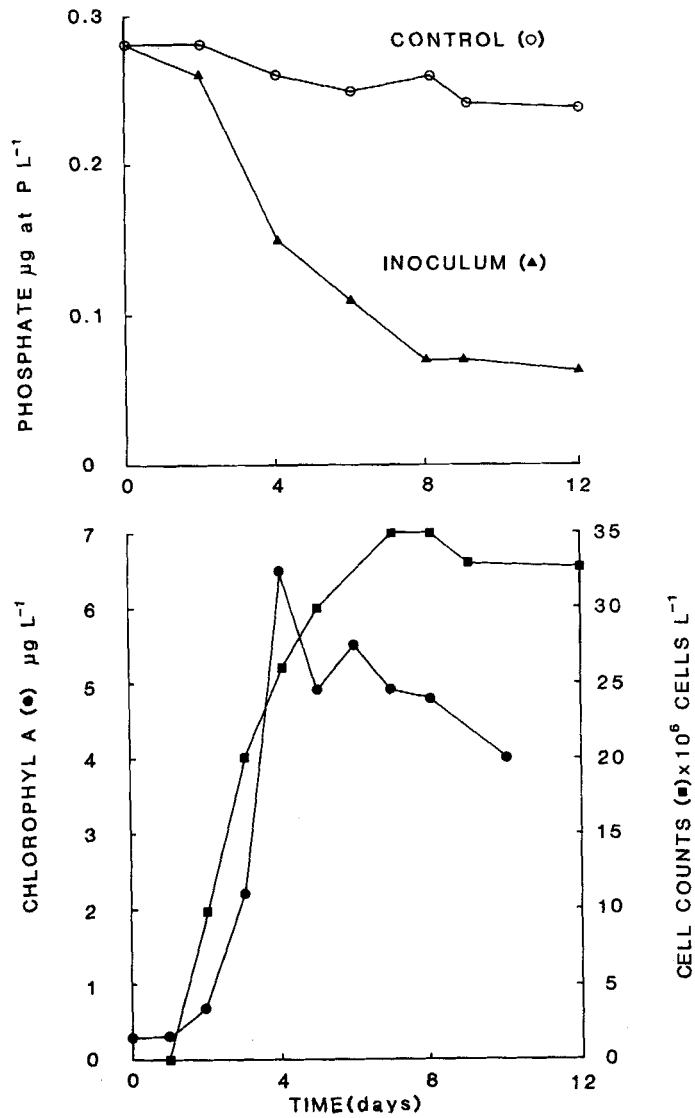

Figure 3 Time-dependent behaviour of reactive phosphate, chlorophyll *a* and cell counts in a culture of *Skeletonema costatum*.

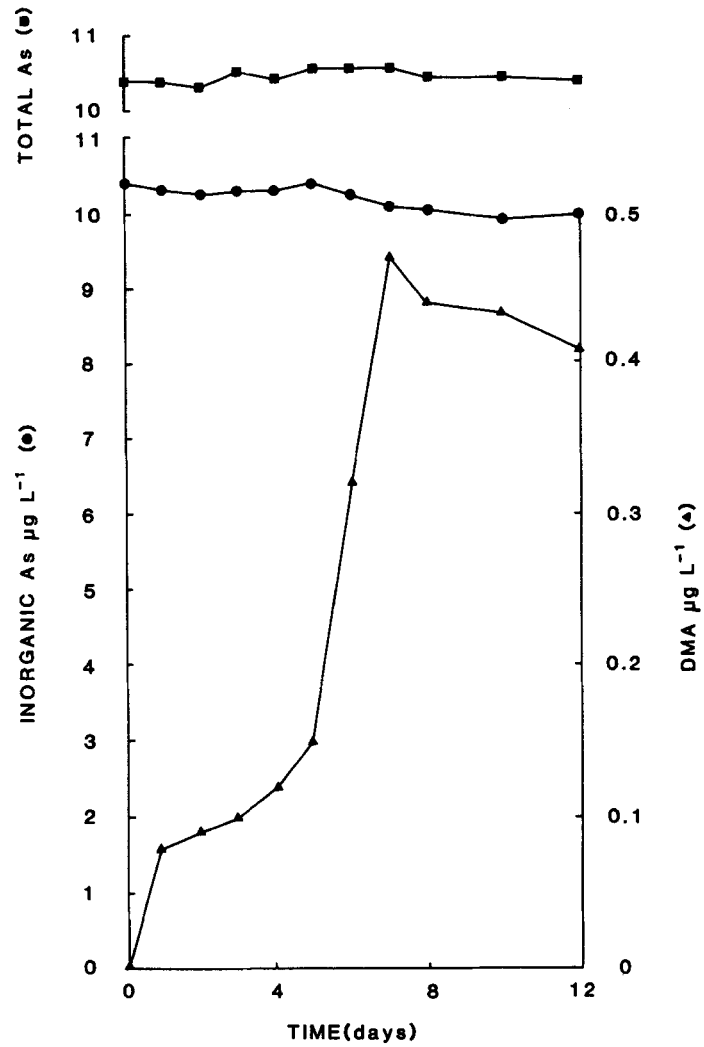

Figure 4 Time-dependent behaviour of the concentrations of dissolved arsenic species (μg dm^{-3}) in a culture of *Skeletonema costatum*.

attained a constant concentration, which was maintained over 7–12 days and which was approximately 4.5% of the total arsenic. The appearance of DMA could be associated with the trend in chlorophyll *a* concentrations, although the maximum chlorophyll *a* concentration was observed on day 4, after which concentrations declined, suggesting that some cells were degrading and chlorophyll *a* was particularly susceptible to this process. Thus, this is strong evidence to suggest that the decomposition of *Skeletonema costatum* cells could be associated with the release of DMA to the water column.

In a similar laboratory study using comparable cell densities of *Skeletonema costatum*, Sanders and Windom[26] observed an increase in cellular arsenic concentrations for arsenic-doped seawater. Furthermore, it was shown that seawater containing an arsenic concentration of 5 μg As dm^{-3} had 20% conversion to DMA by *Skeletonema costatum* over a period of 11 days. However, Sanders and Windom[26] did not report the detection of measurable concentrations of MMA. Sanders[12] also showed that methylated arsenic in Chesapeake Bay was highly correlated with phytoplankton concentrations and, in parti-

cular, the phytoplankton *Chroomonas* sp. appeared to be responsible for the distribution of MMA in the Bay. Apte *et al.*[27] studied the release of arsenic species in the waters of Loch Ewe (northwest Scotland) during a bloom in spring, using an experimental mesocosm (5 m diameter × 17 m depth). The major diatom species was *Skeletonema costatum*, although *Nitzschia delicatissima* and *Thalassiosira* were also present. They observed the release of DMA in mesocosm waters below 3 m, but at depths near 17 m maximum concentrations of DMA were observed. The maximum DMA value of 0.9 μg dm^{-3} was observed after about 18 days, which represented about 60% of the total arsenic concentration of 1.4 μg As dm^{-3}. The Loch Ewe study strongly suggests that methylated arsenic species are released from phytoplankton as they sink through the water column and undergo bacterial decay, with DMA being the first product to appear. This confirms the results presented here because MMA was not detected throughout the course of the 12-day incubation experiment. This could be related to the hydrodynamics of the Tamar Estuary, which has a flushing time between 7 and 12 days,[28] which is similar to the time or the appearance of DMA. This suggests that the estuary water is not present for long enough for MMA generation processes, such as demethylation of DMA or exudation of MMA from phytoplankton, to get underway. Thus, these results suggest that MMA in the waters of the lower Tamar is unlikely to originate from phytoplankton; rather, it comes from another source.

An approximate rate of release of DMA into the water column during the log growth phase is estimated (from Fig. 4) to be 3 ng dm^{-3} h^{-1}. At the stationary phase the total mass of cells in 10 dm^3 was estimated to be approximately 130 mg (dry weight): hence this gives a dry weight release rate for DMA from phytoplankton of 200 μg kg^{-1} h^{-1}. In the natural environment the release rate may be higher than this, because in these experiments the culture was free from bacteria, which could contribute to enhanced decomposition of cells. Direct comparison between the release rate of DMA from macroalgae and from phytoplankton is not possible because the wet weight of the latter is difficult to obtain. However, there are significant contrasts in the estuarine biomass of macroalgae compared with phytoplankton which could go some way towards compensating for the differential release rates.

Porewaters

The sediment samples contained significant concentrations of total arsenic, which increased up-estuary, and acetic-acid-available arsenic ranged from 11% in freshwater to 13% in seawater sediments (see Table 2). In abiotic experiments at 5 °C and 15 °C involving autoclaved sediments, the MMA concentration was 0.06 μg dm^{-3} at the start of the experiment and after 600 h of incubation it was 0.08 μg dm^{-3}. Similarly, the DMA concentration started at 0.08 μg dm^{-3} and it was 0.11 μg dm^{-3} at the end of the incubation period. Thus, the increases in concentration of methylated species were small and could have originated from remnant phytoplankton or macroalgal tissue in the sediment samples.

Figure 5 shows the results from the incubation of freshwater sediments at 5 °C. Both MMA and DMA appeared after an initial delay period of 100 h. More DMA than MMA was observed, and both species reached a steady-state concentration in the overlying water after 250 h. Figure 6 shows the complementary experiment carried out at 15 °C, which demonstrates the appearance of methylated species again after 100 h with more DMA than MMA. At 15 °C the maximum DMA concentrations were twice those at the lower temperature and were reached after 250 h. There were no differences in the maximum MMA concentrations between summer and winter conditions. In the simulations using marine sediments, different behaviour was observed. At 5 °C DMA did not appear above background concentrations for the whole of the 1000 h recording period (Fig. 7). However, after an initial delay period of 350 h, MMA was released into the overlying water, with its maximum concentration being reached after 500 h. When the temperature

Table 2 Total and acetic acid available arsenic in Tamar Estuary sediments (1986)

Site	Distance up-estuary (km)	Arsenic concentration[a] (μg g^{-1}, dry wt)	
		Total arsenic	Available arsenic
Calstock	30	77.9	8.8
Halton Quay	23	63.7	6.8
Cargreen	19	43.7	8.9
Riverside	14	41.9	4.6
St John's lake	7	35.2	4.7

[a] Average of duplicate analyses.

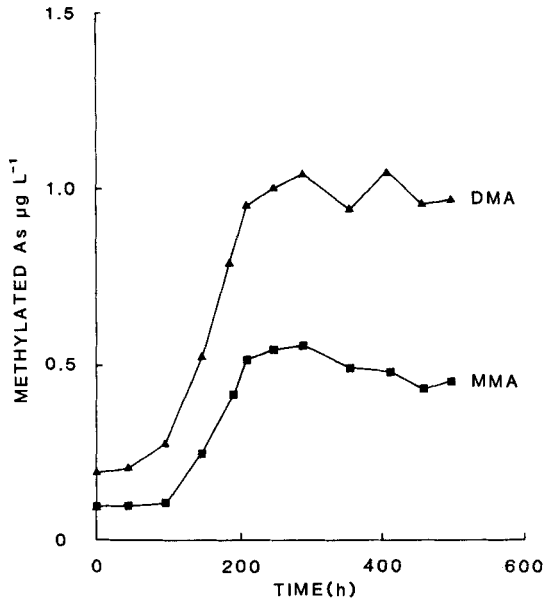

Figure 5 Time-dependent evolution of MMA and DMA ($\mu g\ dm^{-3}$) from freshwater sediments incubated at 5 °C.

was raised to 15 °C MMA appeared after a 100 h delay period and reched its steady state after 200 h (Fig. 8). In contrast, for DMA only a relatively small increase ($<0.5\ \mu g\ dm^{-3}$) was observed after 500 h. The rate-determining step in the appearance of these species in the overlying water may be either kinetically constrained production of a precursor of the methylated compounds in the porewaters, such as reduced arsenic species, or slow diffusion of pre-existing methylated compounds, or a combination of the two processes.

Several trends are apparent from these results. Firstly, more DMA was observed in both freshwater and seawater systems at the higher than at the lower temperature. Furthermore, the freshwater sediments generated methylated species more rapidly than marine sediments. Thus, in freshwater sediments the production of DMA and MMA reached a steady state in 250 h, compared with 500 h for MMA in the equivalent marine sediments. Another marked difference is that in freshwater DMA production exceeds MMA production, while the reverse is the case in the marine sediments. The results from the laboratory experiments support field observations of methylated arsenic species in sediment porewaters, which tend to show more MMA than DMA in marine porewaters, in contrast with freshwater ones.[22] The prevalence of either one or the other of the methylated species may be attributed to the presence of different micro-organisms in each sediment and these results strongly suggest *in situ* methylation. Wong *et al.*[29] reported that the freshwater bacteria *Aeromonas* sp. and *Flavobacterium* sp. produce DMA in preference to MMA. In the Tamar sediments only *Pseudomonas* sp. and marine bacteria of the genus *Bacillus sp.* were positively identified, but only sparse data on the arsenic methylation potential of these species are currently available. However, Shariatpanahi *et al.*[30,31] have shown that *Pseudomonas* sp., doped with sodium arsenate and sodium methylarsonate, yielded methylated species, including MMA and DMA. In

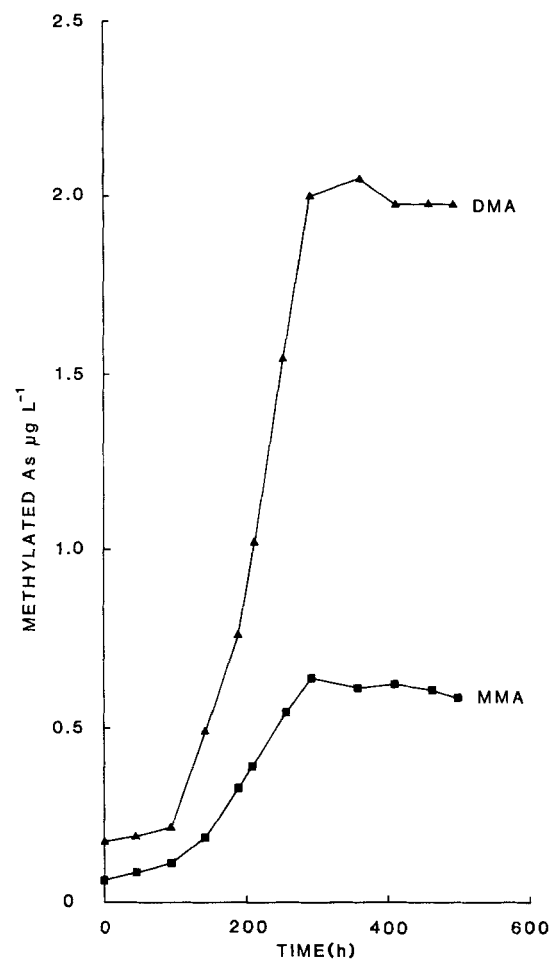

Figure 6 Time-dependent evolution of MMA and DMA ($\mu g\ dm^{-3}$) from freshwater sediments incubated at 15 °C.

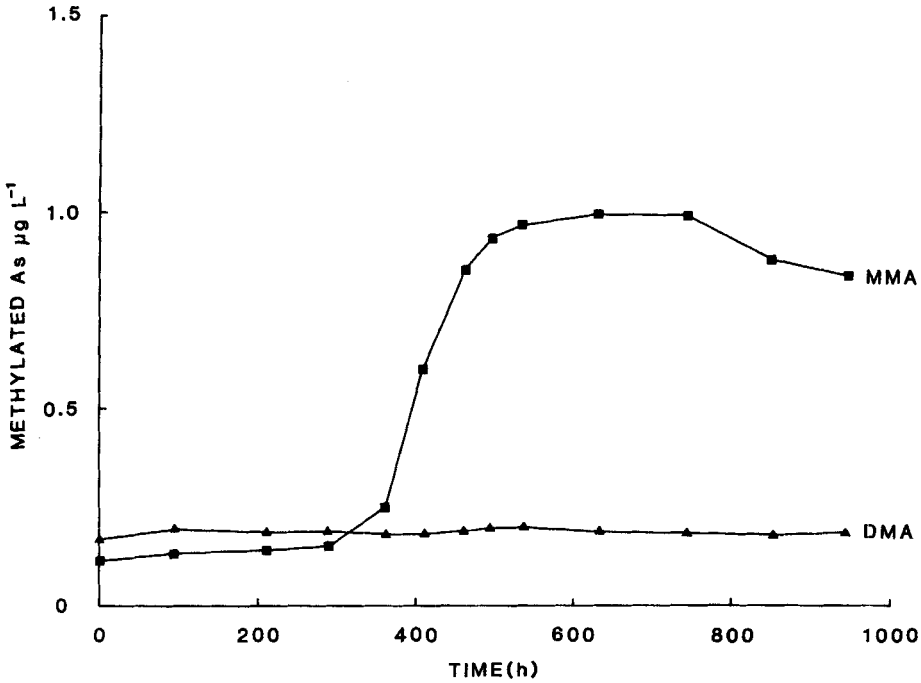

Figure 7 Time-dependent evolution of MMA and DMA ($\mu g\,dm^{-3}$) from marine sediments incubated at 5 °C.

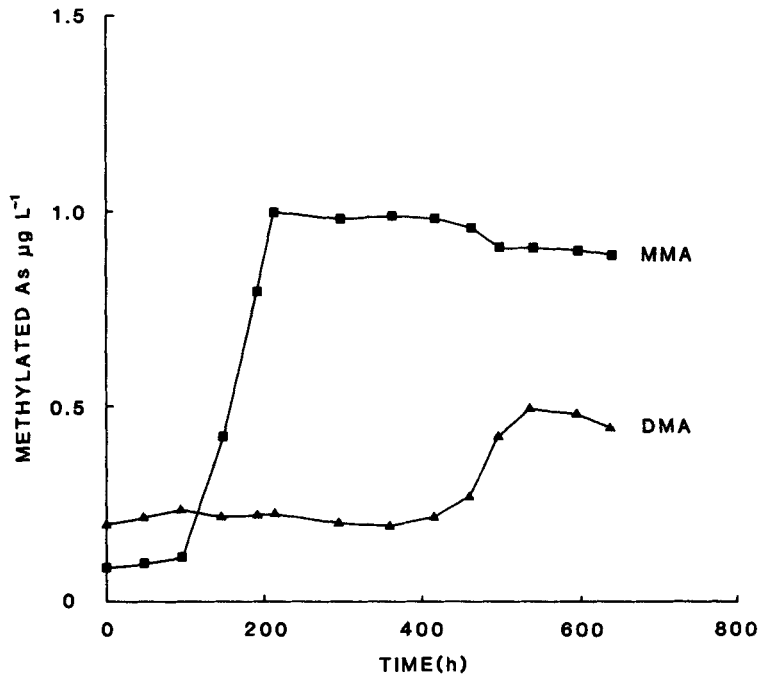

Figure 8 Time-dependent evolution of MMA and DMA ($\mu g\,dm^{-3}$) from marine sediments incubated at 15 °C.

addition, sodium methylarsonate was demethylated to arsenate.[31]

Seasonality in sources of methylated arsenic in the Tamar Estuary

In order to examine the relative magnitudes of the potential sources, first-order flux calculations of the release of methylated arsenic species from the sources to the water column were performed. The contribution from macroalgae in St John's Lake was estimated using a seaweed density of 10 kg m^{-2} (wet weight), estimated from observed distributions. The aerial coverage of macroalgae was approximately 10% of the total sediment area of the lake, 2×10^6 m^2, determined from an Ordnance survey map, which gives a total macroalgal mass of 2×10^6 kg (wet weight). Most of the macroalgae are *Ascophyllum nodosum* with a DMA summer release rate of 3 μg kg^{-1} h^{-1}, which gives a DMA input of 6 g h^{-1}. If the macroalgal mats are covered for 12 h over the tidal cycle (amounting to a water volume of about 10^6 m^3), then the concentration of DMA in the water column could be augmented by approximately 0.08 μg dm^{-3} and MMA by 0.01 μg dm^{-3}. By comparison, in winter the releases of methylated species by seaweed were considerably lower, yielding increases in DMA concentrations of 0.01 μg dm^{-3} and MMA of less than 0.01 μg dm^{-3}.

Flux estimates can also be made for the input of methylated arsenic species from sediment porewaters in St John's Lake. Given a total sediment area of 2×10^6 m^2 and assuming a sediment depth of 0.05 m involved in the porewater infusion mechanism, a sediment volume of 10^5 m^3 results. If 60% of this volume is occupied by porewater, then the volume of sediment interstitial water is 6×10^7 dm^3. Ebdon *et al.*[22] showed that, during summer, porewaters at this site had 0.2 μg dm^{-3}, which amounts to a total of 12 g, of arsenic as MMA in the sedient porewaters of St John's Lake. If this amount of MMA were mobilized into the waters covering St John's lake over a tidal cycle, the MMA concentrations would be enhanced by less than 0.01 μg dm^{-3}. Similarly, DMA concentrations, which in porewaters are typically 0.2 μg dm^{-3} in summer, would also contribute less than 0.01 μg As dm^{-3}. In the winter, however, when MMA porewater concentrations are in the range 0.5–0.7 μg dm^{-3}, water-column MMA concentrations could increase by 0.03 μg dm^{-3}. Porewater DMA concentrations in winter are 0.15–0.20 μg dm^{-3}, which would lead to water-column increases of less than 0.01 μg dm^{-3}.

During spring, phytoplankton blooms grow in the lower Tamar Estuary and as a consequence arsenate is taken up and methylated. The phytoplankton are grazed upon by bacteria which assist in the release of quantities of DMA to the water column. The diatom *Skeletonema costatum* released DMA at a rate of 3 ng dm^{-3} h^{-1} during its log growth phase, which over a tidal cycle yields an increase of DMA of about 0.04 μg dm^{-3}. MMA was not observed because the processes responsible for its production were slow compared with the flushing of water (and phytoplankton) from the estuary.

CONCLUSIONS

This study has attempted to evaluate the relative strengths of three important sources, namely phytoplankton, macroalgae and sediment porewaters, of methylated arsenic species in the lower Tamar Estuary. The data have been summarized in Table 3 in terms of the potential contribution to observed dissolved concentration of the species during summer and winter.

Field observations in St John's Lake during summer show the water column concentrations of DMA[8,9] to be in the range from 0.2–1.3 μg dm^{-3}; most of this DMA has been assumed to originate from phytoplankton. However, the results above show that the contribution to DMA in the water column made by macroalgae could be of the order of 60% and should be incorporated into estuarine arsenic cycles. The porewaters of marine sediments are not significant contributors of DMA (in fact, MMA is preferentially released) to the water column in summer, and in any case the process responds weakly to the seasonal change in tem-

Table 3 Seasonal variation in the relative contributions of methylated arsenic species to the waters of the lower Tamar Estuary, over a tidal cycle

	DMA (μg dm^{-3})		MMA (μg dm^{-3})	
Source	Summer	Winter	Summer	Winter
Macroalgae	0.08	0.01	0.01	<0.01
Phytoplankton	0.04	0	0	0
Sediment porewaters	<0.01	<0.01	<0.01	0.03

perature. Thus, the localized *in situ* processes of phytoplankton and macroalgal degradation and porewater infusions do not fully account for the observed DMA concentrations. Down-estuary transport of DMA to the lower estuary has to be considered and a potentially significant source of DMA are the freshwater sediments, which could provide a significant infusion of DMA during the summer. Observed MMA concentrations in St John's Lake,[8,9] in the summer, were always less than DMA values and were in the range from <0.05 to $0.4\,\mu g\,dm^{-3}$. During the laboratory experiments on a population of diatoms, no evidence was found for the appearance of MMA over a 12-day period, because either MMA is not a primary product of phytoplankton degradation or the chemical lifetime of MMA is short and it is rapidly transformed to arsenate. Inputs of MMA from macroalgae could be significant, together with minor inputs from sediment porewaters. Down-estuary transport of MMA is probably not a significant factor because freshwater sediments release only small quantities of MMA.

In the winter, observed concentrations of DMA in St John's Lake[8] are in the range from 0.10 to $0.25\,\mu g\,dm^{-3}$. The contribution to DMA in the water column by macroalgae is significantly lower than in summer but it could still be the main source (see Table 3). Inputs from the sediment porewaters in the lake are negligible, as is the contribution from down-estuary transport of DMA infusions from freshwater sediments. In contrast, winter concentrations of MMA in the waters of St John's Lake were higher than DMA values and were approximately $0.35\,\mu g\,dm^{-3}$ in February and March 1986. However, the contribution made by porewater infusions of MMA in winter appears to be significant, notwithstanding the delay in its release to the water column, and this source must be taken into account (see Table 3). It is conceivable that some of the porewater MMA could have been the result of demethylation of DMA originating from the decay of phytoplankton and macroalgal tissue following the previous summer.[22]

Although these estimates are based on first-order assumptions, they indicate that sources other than phytoplankton can contribute significantly to methylated arsenic in estuaries.

Clearly, in the summer the important sources are macroalgae and phytoplankton but in winter, when these sources are at a low intensity, the porewater source becomes significant. Better identification of the rates and extents of these processes is called for in the development of predictive models of estuarine arsenic behaviour.

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