APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 6, 213–219 (1992)

Bioaccumulation of arsenic and its fate in a freshwater food chain

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Accumulation, biomethylation and excretion of arsenic by an autotrophic freshwater alga, and the transport and transformation of the arsenic in the freshwater food chain [alga (autotroph) – moina (planktonic grazer) or shrimp (herbivore) – guppy (carnivore)] were investigated.

These experimental results lead to the conclusion that total arsenic concentrations in organisms after accumulation from foods decreased one order of magnitude per elevation of the trophic level and biomethylation of the arsenic increased successively with an elevation in the trophic level. Predominant methylated arsenic species in moina and guppy were dimethyl- and trimethyl-arsenic compounds, respectively. Shrimp accumulated dimethyl- and trimethyl-arsenic compounds in nearly equivalent quantities. No or little monomethylarsenic compound was detected either in herbivores or carnivores.

Keywords: Arsenic, methylation, algae, bioaccumulation

INTRODUCTION

Organoarsenic compounds in marine organisms and the transport of arsenic via the food chain have been investigated by many researchers.[1–4] However, only a few experiments have been conducted in freshwater systems. Giddings[5] reported bioaccumulation ratios (concentration factors) of arsenic accumulation in artificial lake microecosystems (70 dm^3) by three trophic levels of freshwater organisms (phytoplankton–zooplankton–snail) as being 965, 192 and 11, respectively. Direct arsenic accumulation from the aqueous phase and indirect accumulation via the food chain, however, cannot be distinguished from these data. Experimental data so far obtained do not give information about biotransformation of arsenic between various chemical forms during passage through the food chain.

Previously we reported arsenic-resistance of marine microorganisms[6,7] and freshwater microalgae,[8–12] and their arsenic accumulation,[8–10] biomethylation[11,12] and excretion.[12]

The transformation of inorganic arsenic compounds in the freshwater food chain starting from freshwater microalgae through grazers (zooplankton: *Moina* sp.) to carnivores (goldfish: *Carassius* sp.[13] or guppy: *Poecilia* sp.[14]) have been reported. These experimental results showed that the total arsenic concentration decreased by one order of magnitude, and the relative concentration of methylated arsenic to the total arsenic, on the contrary, increased successively with an elevation in the trophic level.

This paper presents experimental results both on the metabolism of methylated arsenic compounds by freshwater alga (*Chlorella* sp.) and on the transformation of inorganic arsenic obtained with a similar food chain but with the shrimp as a grazer.

EXPERIMENTAL

Culture of organisms

Autotrophic green algae

Chlorella vulgaris Beijerinck var. *vulgaris* which had been isolated from an arsenic-polluted environment by the authors[8] was used as an algal sample. For the change of growth, accumulation, biomethylation and excretion of arsenic with the passage of time, a suspension (4 cm^3, 120 mg dry mass) of *Chlorella* sp. was placed in a modified Detmer medium[1] (20 dm^3) containing 100 mg dm^{-3} arsenic [as elemental arsenic for Na_2HAsO_4, abbreviated as As(V)]. The culture was kept at 25–30 °C under constant aeration (200 cm^3 min^{-1}), illumination (4000 lux) and

0268–2605/92/020213–07 $05.00

Received 9 July 1991
Accepted 18 December 1991

germ-free conditions for 20 days. The cell suspension (few mg dry mass) was harvested every two days, and the cells were separated by centrifugation; the cells and supernatant were analyzed for total arsenic.

For studying the transformation of inorganic arsenic, *Chlorella* cells (6 mg dry mass) were placed in a modified Detmer medium (containing 100 mg dm^{-3} As(V)). The culture was carried out similarly as mentioned above, but for seven days.

Planktonic grazer

Moina macrocopa was obtained from Mr T Oyama of Kagoshima Prefectural Fishery Experiment Station. *Moina* sp. was fed with arsenic-free bread yeast ('Super camellia', dry yeast, manufactured by Nissin Seifun Co., Japan) in an aerated diluted Detmer medium (one part medium, nineteen parts distilled water).

Herbivorous shrimp

The shrimp samples were collected from a natural clear stream in Kagoshima prefecture. The shrimp was identified as *Neocaridina denticulata* by Dr Masanori Satoh of Kagoshima University. *Neocaridina* sp. was fed with a basic diet ('Tetrafin', manufactured in West Germany) in aerated diluted Detmer medium (one part medium, forty-nine parts distilled water).

Carnivorous guppy

Poecilia reticulata was obtained from Mr T. Oyama. *Poecilia* sp. was fed with 'Tetrafin' in aerated diluted Detmer medium (one part medium, nineteen parts distilled water). Adult guppies were used for the tests.

Determination of total and methylated arsenic compounds

For the determination of total arsenic, the dry cells (10–20 mg) were mixed with 50% aqueous $Mg(NO_3)_2$ (2 cm^3); the mixture was dried and mineralized by heating at 550 °C for 6 h. The mineralized samples were dissolved with 10 mol dm^{-3} HCl (10 cm^3), 40% KI (1 cm^3) was added, the solution was extracted twice with $CHCl_3$ (5 cm^3) and the $CHCl_3$ phase was then back-extracted with water (2 cm^3). Total arsenic was determined in the water phase by graphite furnace–atomic absorption spectroscopy (GF-AAS). Conditions for As determinations by GF-AAS are as follows: injection volume 20 μdm^3 drying 150 °C 10 sec; ashing 650 °C 10 sec; atomization 2200 °C 5 sec. Detection limits for total arsenic was 5 ng in 20 μdm^3.

For the determination of methylated arsenic compounds, the dry cells (*ca* 10 mg) were digested with 5 cm^3 of 2 mol dm^{-3} NaOH at 90–95 °C for 3 h, using an aluminum heating block. Methylated arsenic compounds in the digest were reduced with sodium borohydride ($NaBH_4$) to the arsenic compounds. The arsine gases were flushed by helium and frozen out in a liquid-nitrogen U-trap. Upon warming the U-trap the arsines were borne out of it successively and were passed through a quartz tube atomizer and determined on an atomic absorption spectrometer.[11]

Sodium methylarsonate, dimethylarsinic acid and arsonobetaine were used as authentic samples for monomethyl-, dimethyl-, and trimethylarsenic compounds (abbreviated as MMA, DMA and TMA), respectively.

On the hot base digestion, the methylated arsenic compounds are not degraded into inorganic arsenic.[15] Arsenobetaine[16,17] and dimethylarseno/ribose derivatives[16] are quantitatively converted to trimethylarsine oxide and dimethylarsinate on the hot base digestion, and hydrided to trimethylarsine and dimethylarsine on treatment with borohydride,[19] respectively. The arsenobetaine and arsenosugars have been generally found in marine animals and plants, respectively.

On the other hand, arsenocholine, tetramethylarsonium and some other methylated arsenic compounds were not quantitatively converted to the corresponding methylarsenic compounds on the hot base digestion.[16,17] These compounds were found in nature but not in general.

The authors assumed that all the methylarsenic compounds present in the freshwater organisms in this paper are measured by the hot base digestion-hydride generator method.

RESULTS AND DISCUSSION

Change in growth of *Chlorella* sp. and accumulation of arsenic by *Chlorella* cells with the passage of time

Algal growth and arsenic concentrations in both cells and medium at set culture times are plotted in Fig. 1, in which ○ and □ represent the growth of cells (g dry mass per dm^3 medium) and the observed arsenic concentration in the cells (mg As per g dry mass) respectively, and △ are points calculated from observed analytical values. The solid curves y_1, y_2 and y_3 are obtained by the semi-theoretical equations [1]–[3]

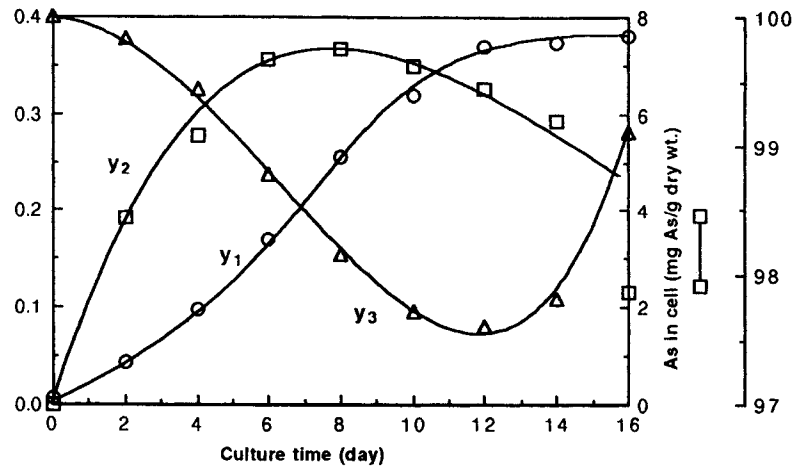


Figure 1 Growth curve (cell concentration, g dry wt./dm^3) (○) for *Chlorella* sp. and arsenic concentration in cell (□) and in medium (mg As/dm^3) (△), and the theoretical curves (solid lines) in the germ-free system.

$$dy_1/dt = p \times y_1 \times (q - y_1)/q \quad [1]$$

$$dy_2/dt = k_1 \times y_3 - k_2 \times y_2 - k_3 \times t \quad [2]$$

$$y_3 = y_{3,0} - y_1 \times y_2 \quad [3]$$

where y_1 is growth (g cell per dm^3) y_2 is arsenic concentration in the cell, y_3 is arsenic concentration in the medium (mg As per dm^3 medium), p is the growth parameter, q is the cell concentration in the stationary growth phase, and k_1, k_2 and k_3 are constants. Equation [1] is well known as the logistic equation. Equations [2] and [3] are introduced by the present authors. The authors first assumed that arsenic concentration in the cell (y_2) increases in proportion to the arsenic concentration in the medium (y_3) and decreases with an increase in y_2 from the observed values (□) shown in Fig. 1. The calculated values based on the assumption, however, did not agree with the observed values. So, the authors then added the correction term presenting y_2 as decreasing with time in Equation [2]. Equation [3] was obtained from subtraction of the product of y_1 and y_2 from the initial arsenic concentration ($y_{3,0}$ in the medium).[18]

From Fig. 1, the observed growth curve (○) is found to agree with the theoretical curve, y_1. Agreement was also obtained in the previous experiments using algae (*Phormidium* sp.)[12] and bacteria (*Pseudomonas putida*).[20] The observed values, □ and △, are also found to agree with the theoretical curves, y_2 and y_3. These results mean that the assumption mentioned above is reasonable.

These results lead to a conclusion that arsenic concentration by *Chlorella* sp. is greatest during the logarithmic growth phase corresponding to the inflection point of the growth curve and that arsenic concentration in the cell decreases after the stationary phase, because the rate of excretion exceeds the rate of uptake of arsenic. Arsenic concentration in the surrounding medium consequently, has a minimum value at the beginning of the stationary growth phase. Clearly the algal cells take up arsenic at rates dependent on the culture time. At the beginning of the growth phase the take-up rate exceeds the excretion rate; at the maximum cell concentration of arsenic in the growth curve the rates are equal, and at the stationary phase the excretion rate is higher than the take-up rate!

Change in methylated arsenic compounds in cells and in water

Chlorella sp. was cultured in modified Detmer medium (20 dm^3) containing 9 mg As(V) dm^3 for 20 days and methylated arsenic compounds accumulated in the cell and excreted in the medium were determined every two days. The experimental results are summarized in Table 1. In Table 1, IA values were obtained from subtraction of the sum of methylated arsenics (MMA + DMA + TMA) from the total arsenic concentration.

Table 1 shows that monomethyl- and dimethylarsenic compounds were produced in the algal cell in the initial stage of the growth and that both concentrations fell with growth time. Production

Table 1 Arsenic compounds accumulated in *Chlorella* cells and excreted in the medium

Culture time (day)	Arsenic in *Chlorella* (μg As g^{-1} in dry mass)					Arsenic in medium (μg As dm^{-3} medium)			
	Total	IA	MMA	DMA	TMA	Total	MMA	DMA	TMA
0	0	0	0	0	0	9000	0	0	0
2	1140	1100	15	20	tr	8300	—	—	—
4	2290	2270	tr	16	0.1	7900	—	—	0.3
6	3230	3200	12	15	3.9	7700	—	—	1.5
8	3410	3380	10	13	5.0	8200	—	—	4.9
10	3750	3720	9	11	6.1	7600	—	—	13.4
12	3510	3480	9	11	5.4	6900	—	tr	tr
14	2600	2570	6	11	4.6	6700	tr	0.3	0.2
16	2520	2500	tr	10	4.6	7000	—	—	tr
18	1550	1530	2.2	9	5.3	7200	tr	0.7	—
20	1950	1930	3.5	9	4.6	8000	tr	0.5	tr

Abbreviations: IA, non-methylated arsenic; MMA, monomethylarsenic; DMA, dimethylarsenic; TMA, trimethylarsenic; tr, trace; —, not detected.

of trimethylarsenic compounds was found to appear in the cells a few days after that of monomethyl- and dimethyl-arsenic compounds and the concentration of trimethylarsenic in the cell became constant at the end of the exponential growth phase.

On the other hand, methylated arsenic compounds were also excreted by the algae into the surrounding medium phase. The predominant methylated arsenic species found in the medium was trimethylarsenic.

Accumulation of arsenic by the shrimp (*Neocaridina denticulata*) from the water phase

Five groups of shrimps were each fed in a 1-dm^3 aerated diluted modified Detmer (1:50) medium containing 0.1, 0.2, 0.3, 0.5, 1.0, 1.5 and 2.0 mg As(V) dm^{-3} for seven days. The separated shrimps were washed with distilled water, heated at 60 °C to dryness and analyzed for total arsenic and methylated arsenic compounds.

The shrimp did not survive in a medium with 2.0 mg dm^{-3} of arsenic as Na_2HAsO_4. Thus, shrimps are not as arsenic-tolerant as moina (*Moina macrocopa*),[13] guppies (*Poecilia reticulata*)[14] and goldfish (*Carassius carassius auratus*);[13] those survived in media with less than 3.0, 15 and 25 mg As(V) dm^{-3}, respectively. Experimental results obtained from surviving shrimps are summarized in Table 2.

Table 2 shows that accumulation of arsenic by shrimps from the medium increased with an increase of the arsenic concentration in the medium up to 1.0 mg As dm^{-3}. The total concentration of arsenic accumulated by shrimps from the water phase was comparable with that *Moina* sp. (18 μg As g^{-1})[13] but lower than that by goldfish (51 μg As g^{-1}).[13]

Table 2 Accumulation and methylation of inorganic arsenic by shrimps (*Neocaridina* sp.) from the arsenic-containing medium

As(V) in water (mg As dm^{-3})	Concentration of arsenic in shrimp, μg As g^{-1} dry base (%)				
	Total	IA	MMA	DMA	TMA
0.1	18.9(100)	15.9(84.1)	—	1.9(10.1)	1.1(5.8)
0.2	18.5(100)	14.9(80.5)	tr	1.9(10.3)	1.7(9.2)
0.3	19.8(100)	17.3(87.4)	—	1.4(7.1)	1.1(5.5)
0.5	22.6(100)	15.4(68.1)	tr	2.6(11.5)	4.6(20.4)
1.0	33.2(100)	30.2(91.0)	tr	1.7(5.1)	1.3(3.9)
1.5	31.6(100)	27.9(88.3)	tr	2.2(7.0)	1.5(4.7)

Abbreviations: as shown in Table 1.

Table 3 Accumulation of arsenic by shrimps (*Neocaridina* sp.) from food (*Chlorella* sp.)

Organism	Feeding time (days)	Concentration of arsenic in organism, μg As g^{-1} dry base (%)				
		Total	IA	MMA	DMA	TMA
Food[a]		1940	1932(99.6)	1.4(0.007)	6.6(0.33)	tr
Shrimp						
	2	16.9	15.6(92.2)	—	1.0(6.0)	0.3(1.8)
	4	13.8	11.9(86.1)	—	1.3(9.7)	0.6(4.2)
	6	26.3	24.3(92.2)	—	1.0(3.9)	1.0(3.9)
	8	31.8	29.2(91.8)	—	1.8(5.8)	0.8(2.4)
	10	28.2	25.4(90.1)	—	1.4(5.0)	1.4(5.0)
	12	23.7	20.5(86.4)	—	1.6(6.8)	1.6(6.8)
	14	22.3	19.1(85.6)	—	1.2(5.4)	2.0(9.0)
	16	21.8	18.7(85.8)	—	1.6(7.3)	1.5(6.9)
	18	26.3	22.9(87.1)	—	1.6(6.1)	1.8(6.8)
	Average[b]	23.45			1.39(5.9)	1.22(5.2)

Abbreviations: as shown in Table 1.
[a] Live *Chlorella* cells. [b] Average of arsenic concentrations in shrimp.

Direct methylation of inorganic arsenic accumulated by shrimps from the water phase was found. About 10–30% of the total arsenic accumulated was methylated. These percentages were much larger than those obtained from algal experiments[8–12] but comparable with those from *Moina* sp.[13] Monomethylated arsenic was detected only in traces or not at all.

Accumulation of arsenic by shrimp (*Neocaridina denticulata*) from food (*Chlorella vulgaris*)

Algal cells were cultured in modified Detmer medium containing 100 mg As(V) dm^{-3} for seven days and the arsenic-accumulated algal cells were fed to 30 shrimps for 18 days. The living algal cells were fed to shrimps every day in an appropriate quantity for the consumption. Three shrimps were harvested every two days and analyzed for arsenic. The experimental results are summarized in Table 3. Relative concentrations of methylated arsenic in *Chlorella* sp. and *Neocaridina* sp. are illustrated in Fig. 3.

Table 3 shows that total arsenic accumulated by shrimps increased with an increase in feeding time up to the eighth day and did not increase thereafter. On the other hand, the concentration of methylated arsenic compounds in shrimps (MMA + DMA) continuously increased. No monomethylarsenic compound was detected in

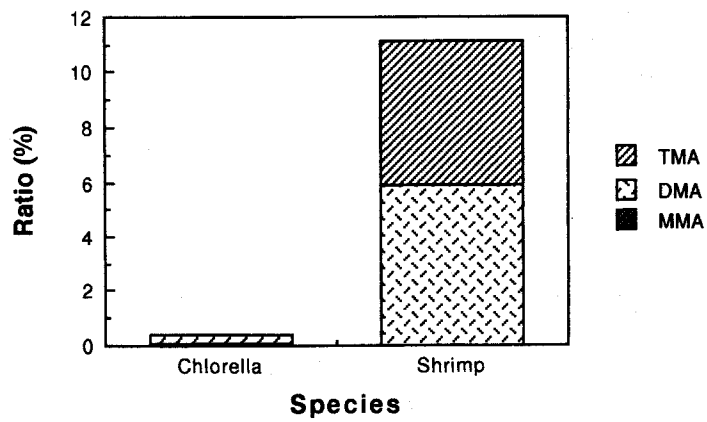


Figure 2 Relative concentration of methylated arsenic accumulated by *Chlorella* sp. from medium and by shrimp from *Chlorella* sp. Ratio is % As species (TMA, DMA or MMA) in the organism compared with total As in the organism. Average values for shrimp shown in Table 3 are illustrated in this Figure.

Table 4 Biotransformation of arsenic in food chain of alga (*Chlorella* sp.)–moina (*Moina* sp.)–guppy (*Poecilia* sp.)

Organism	Concentration of As in organism, μg As g^{-1} (% of total As)				
	Total	IA	MMA	DMA	TMA
Chlorella sp.	1037	1031(99.4)	0.8(0.08)	5.0(0.50)	0.3(0.03)
Moina sp.	36.9	27.5(74.5)	tr	9.3(25.4)	0.02(0.05)
Poecilia sp.	8.5	1.5(17.6)	tr	0.1(1.2)	6.9(81.2)

Abbreviations: as shown in Table 1.

shrimps, whereas it had been contained in the food (*Chlorella*).

Concentration of the dimethylarsenic compounds was nearly constant but the trimethylarsenic compound rose gradually relative to total arsenic in the organism. Figure 2 shows that methylated arsenic in shrimp is much greater than that in algae.

These results show that inorganic and monomethylated arsenic compounds in food (*Chlorella* sp.) were digested by shrimps and biomethylated to dimethyl- and trimethyl-arsenic compounds, respectively. A nearly steady total arsenic concentration, and a gradual increase in trimethylarsenic compound in shrimps after the sixth day of feeding, suggest that the shrimps have a limited capacity for arsenic accumulation and that excess arsenic intake is methylated and excreted to the water phase. The predominant chemical species of arsenic excreted is estimated as trimethylarsenic compounds from the experimental results shown in Table 1.

Biotransformation of arsenic in the food chain of autotrophic alga (*Chlorella* sp.)–zooplanktonic grazer (*Moina* sp.)–carnivorous adult guppy (*Poecilia* sp.)

In a previous paper, 82% (trimethyl) and 3% (dimethyl) arsenic were methylated in the guppy (*Poecilia* sp.). In that earlier paper in the same food chain experiment system, young guppies (1.5 cm long and 10 mg dry mass each) were tested.[14] In the present paper, adult guppies (about 5 cm long and 100 mg dry mass) were tested. The other experimental conditions were basically as follows.

Chlorella cells were cultured in modified Detmer medium containing 100 mg As(V) dm^{-3} for seven days, the arsenic-accumulated cells were harvested and rinsed with the arsenic-free medium. Few thousand *Moina* sp. (about 10 mg dry mass) in 10 dm^3 aerated diluted modified Detmer medium were fed with the living *Chlorella* cells for seven days. A part of the moina (about 2 mg dry mass) fed for seven days were analyzed for arsenic. The rest were fed continuously as living bait for guppy for another seven days with the algae in the same way.

Two guppies (*Poecilia reticulata*) in the aerated diluted Detmer medium were fed separately for seven days with the arsenic-accumulated *Moina* sp., and then collected, rinsed with distilled water and analyzed for arsenic.

The experimental results are summarized in Table 4. Relative concentrations of chemical species in the organisms are shown in Fig. 3.

Table 4 shows that total arsenic concentration in the organisms decreased by one order or more of magnitude successively with an elevation in the trophic level. Figure 3 shows that relative concentrations of non-methylated arsenic species decreased, and methylated arsenic increased dramatically, with an elevation of the trophic level. No monomethylarsenic was detected either in *Moina* sp. or *Poecilia* sp., and dimethylarsenic and trimethylarsenic were the predominant methylated arsenic species, respectively. Similar results were reported in the previous paper.[14] Young guppies (which were tested in the previous paper[14]) digested *Moina* sp. having 75.6 μg As g^{-1} (total) and 9.4 μg As g^{-1} (12.4%) dimethylarsenic and transformed the arsenic compounds to 5.6 μg As g^{-1} (total), 0.1 μg As g^{-1} (3%) dimethylarsenic and 4.6 μg As g^{-1} (82%) trimethylarsenic. The adult guppies in the present paper were found to have a similar metabolic mechanism to the young ones.

Numerous researchers on arsenic species in marine organisms reported that the majority of arsenic accumulated in the organisms are without exception in organic forms, that dimethylarsenic

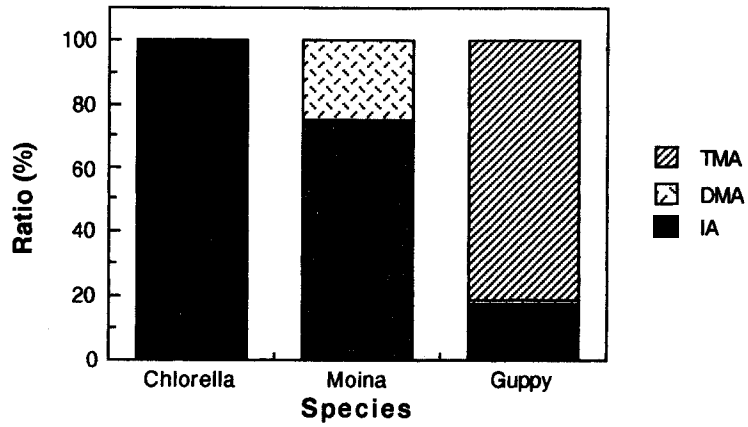
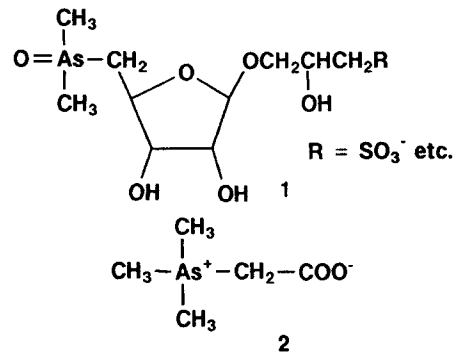


Figure 3 Relative concentration of arsenic accumulated via the food chain.

compounds **1** were found in grazers[21] and that trimethylarsenic compounds such as arsenobetaine **2** were found in fishes and crustacea.[22]



The results obtained from marine organisms, and our results from freshwater organisms, resemble each other well in that herbivorous and carnivorous organisms accumulate predominantly dimethylarsenic and trimethylarsenic, respectively.

Acknowledgements The authors are sincerely grateful to Dr M Satoh of Kogoshima University, Department of Biology, for identification of *Neocaridina denticulata* and Mr T Oyama of Kagoshima Prefectural Fishery Experiment Station for gifts of *Moina* sp. and *Poecilia* sp.

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