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Arsenic species contents at aquaculture farm and in farmed mouthbreeder (*Oreochromis mossambicus*) in blackfoot disease hyperendemic areas

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Abstract

A study was conducted to measure the arsenic species in farmed mouthbreeder (*Oreochromis mossambicus*) and culture ponds in water in blackfoot disease (BFD) hyperendemic areas in Taiwan. The relationships between arsenic species of aquaculture ponds and farmed fish were also explored. Biota samples were extracted with methanol/water (1/1, v/v) using a Soxhlet extraction apparatus. The concentrations of arsenite As (III), arsenate As (V), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) of extracts were measured by high-performance liquid chromatography (HPLC) linked to a hydride generator and atomic absorption spectrometry (HG-AAS). Moreover, arsenobetaine (AB) was analyzed by HPLC linked to ultra violet (UV) and HG-AAS. Concentrations of arsenic species were determined in 68 mouthbreeder (*O. mossambicus*) samples and 21 culture ponds from Putai and Yichu Townships of Chiayi County and Hsuehchia and Peimen Townships of Tainan County. The mean arsenic levels of culture ponds in Putai, Yichu, Hsuehchia, and Peimen were 75.8, 15.1, 14.4, and 221.0 μg/l, respectively. The water of culture ponds was dominated by As (V). The inorganic arsenic percentage of fish (7.4%) was higher than that reported by other seafood surveys. Except for the MMA and As (III) levels, As (V), DMA, AB, and total arsenic levels in fish significantly increased with inorganic and total arsenic concentrations of the pond water. Inorganic arsenic species are more toxic than methyl arsenic species. Therefore the effect of inorganic arsenic species might result in a greater number of adverse health effects to the general public. It is of importance to evaluate the inorganic arsenic levels of farmed seafood in arsenic-contaminated areas.

Keywords: Farmed mouthbreeder; Arsenic species

1. Introduction

Arsenic concentrations found in natural waters range from less than 0.5 μg/l to more than 5000 μg/l. Extreme concentrations are rare but are most frequently found in groundwater (Smedley et al., 2002). High levels of arsenic in well water have been associated with blackfoot disease (BFD) (Ch'i et al., 1968). BFD prevalence rates in Putai, Yichu, Hsuehchia, and Peimen were 8.8, 6.5, 11.6, and 18.9 per 1000 people, respectively (Tseng, 2002). In hyperendemic BFD areas in southwestern Taiwan, total arsenic levels in well waters range from 470 to 897 μg/l. Ninety-five percent of total arsenic are inorganic [As (III) and As (V)] where the predominant arsenic species is As (III) (Chen et al., 1994). Our previous studies illustrated that long-term exposure to inorganic arsenic from drinking water had a significant dose-response relationship with mortality from skin, lung, liver, bladder, kidney, and prostate cancers (Chen et al., 1986, 1992). In addition, cardiovascular diseases,

Abbreviations: BFD, blackfoot disease, As (III), arsenite, As (V), arsenate, MMA, monomethylarsonic acid, DMA, dimethylarsinic acid, AB, arsenobetaine, HPLC, high-performance liquid chromatography, HG-AAS, hydride generator and atomic absorption spectrometry, UV, ultra violet.

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such as ischemic heart disease (Chen et al., 1996; Hsueh et al., 1998b), coronary heart disease (Wu et al., 1989), cerebrovascular accidents (Chiou et al., 1997), carotid atherosclerosis (Wang et al., 2002), hypertension (Chen et al., 1995a; Rahman et al., 1999), as well as diabetes mellitus (Lai et al., 1994; Tseng et al., 2000), are also highly associated with long-term ingestion of drinking water with high levels of arsenic. Since arsenic is epidemologically associated with diseases or cancer, a tap water supply system was implemented in the study villages in the early 1970s. Artesian well water was no longer used for drinking and cooking after the mid-1970s. However groundwater is still used for aquaculture.

Naylor et al. proposed that global production of farmed fish more than doubled in weight and value over the past 15 years. Fish produced from farming activities currently account for over one-quarter of all fish directly consumed by humans. As the human population continues to grow, the reliance on farmed fish production as an important source of protein will also increase (Naylor et al., 2000). Farmed fish is an important economic resource in the southwestern coastal region of Taiwan. Over 50% of farmed mouthbreeder (*Oreochromis mossambicus*) is produced in Tainan and Chiayi Counties of Taiwan (Fisheries Administration ROC, 2000).

It has been known for years that the arsenic concentration in marine organisms is considerably greater than in the surrounding water (Lunde, 1969). Arsenic is mobilized by dissolution in water, with aquatic and soil sediment concentrations being controlled by a variety of input and removal mechanisms (Cullen et al., 1989). There are many arsenic species found in marine organisms. Arsenobetaine was first identified in the Western rock lobster *Panulirus longipes cygnus* in 1977 (Edmonds et al., 1977). The predominant arsenic species present in fish is arsenobetaine (Maher et al., 1999), which is quickly excreted from human bodies and is considered harmless (Cullen et al., 1989). The toxicity of each arsenic species differs significantly according to its oxidation state and/or organic substituents. Inorganic arsenic species are more toxic than methyl arsenic species. The order of lethal doses (LD50) of arsenic species in mice by oral administration is arsenobetaine (10 g/kg) > MMA (1.8 g/kg) > DMA (1.2 g/kg) > As (III) (0.03 g/kg) (Shiomi, 1994). A previous study assessed the effect of seafood intake on human health, and assumed that 10% of total arsenic was inorganic (Han et al., 1998). However, the distribution patterns of organic arsenic species, such as AB, arsenocholine, MMA, and DMA, showed great diversity among various seafoods (Suner et al., 2002). Hence, in order to accurately assess the risk profile, individual arsenic species in seafood must be analyzed.

This is the first study conducted in a BFD hyperendemic area to determine the arsenic species levels in farmed fish and in water from culture ponds. We also examined the associations among each arsenic species, including As (III), As (V), MMA, DMA, arsenobetaine, as well as total arsenic levels in both fish and water.

2. Materials and methods

2.1. Reagents

Deionized water (18 M Ω cm) was used for the preparation of reagents and standards. All glassware was soaked in 10% nitric acid for 24 h, then washed with tap water and rinsed three times with deionized water before use. The stock standard solutions of arsenite, MMA, and DMA were prepared by dissolving appropriate amounts of commercially available salts in water: arsenite [sodium arsenite] (Chem Service); MMA [monosodium acid methane arsonate, 97.5%, CH_4AsNaO_3 * 1.5 H_2O] (Chem Service); and DMA [dimethylarsenic acid sodium salt trihydrate, 97%, $C_2H_6AsNaO_2$*3H_2O] (Merck). The arsenate solution (1000 mg/l) was from Merck. Commercially available arsenobetaine (Fluka) was prepared by dissolving it in appropriate amounts of water.

2.2. Farmed fish and pond water sampling

68 mouthbreeder a (*O. mossambicus*) from 21 aquaculture farms were collected from BFD endemic areas of Taiwan including Putai and Yichu Townships of Chiayi County and Hsuehchia and Peimen Townships of Tainan County. Fish samples and pond water were both collected at the same time. All fish samples were frozen for transportation to the laboratory and stored at −20 °C until they were dissected. Fish mass, width, and total length were measured for each fish. In fish, specimens of dorsal muscle were minced and blended to obtain a homogeneous sample. A portion of homogenized sample was freeze-dried for 36 h and prepared for analysis of arsenic species and total arsenic.

2.3. Analysis of total arsenic

2.3.1. Sample digestion

About 0.5 g of homogenized freeze-dried samples and 25 ml of 65% nitric acid were added to a flask. It was boiled and decanted to allow gases to pass through a condenser and digested 12 h until the solution was clear. Pond water was digested with 65% nitric acid (1/1, v/v) and over-night before the total arsenic analysis.

2.3.2. Determination of total arsenic by HGAAS

Using an electro-thermal atomic absorption spectrometer (AAS, Perkin-Elmer AA100) and hydride generation (HG) system (Perkin-Elmer FIAS-400), and

0.5% $NaBH_4$ in 0.25% NaOH and 1N HCl were added to 200 μl of a digested sample to reduce arsenic to arsine, then the total arsenic concentration was determined. The total arsenic levels of digested sample that pass through the UV light or not were similar (data not shown).

2.4. Arsenic species analysis

2.4.1. Sample extraction and purification

1.0∼1.5 g of freeze-dried fish dorsal muscle and 150 ml of methanol/water solution (1/1, v/v) were placed into a Soxhlet extraction apparatus modified from Gomez-Ariza et al. (Gomez-Ariza et al., 2000) and extracted for 16 h. A methanol removal extraction tube was designed to remove the methanol when the extraction was finished. Following methanol solution removal, the extract was freeze-dried to a powder and redissolved in 10 ml of deionized water. The redissolved liquids were passed through C_{18} cartridges as a purification procedure. On the other hand, pond water was passed through a 45-μm filter as a clean-up procedure before arsenic species were determined.

2.4.2. Determination of arsenic species

As (III), As (V), MMA, and DMA analytical methods followed closely from our previous study (Hsueh et al., 1998a). 200 μl of treated pond water and fish muscle extract were used to separate As (III), As (V), MMA, and DMA by HPLC (Hitachi 7110, Naka, Japan) equipped with an anion column (Machey-Nagel, Nucleosil, 10 μm, 250×4.6 mm), which was linked to HG (FIAS 400 Perkin-Elmer Shelton, USA)–AAS (AA100 Perkin-Elmer Shelton, USA). The mobile phase was a 25 mM phosphate buffer solution (pH = 5.0) and the flow rate of pump was 1.5 ml/min. As (III), As (V), MMA, and DMA chromatogram of standard solution and fish muscle was shown in Fig. 1(A1) and (A2). The elution order was As (III), DMA, MMA, and As (V). There are an unknown peak between As (III) and DMA.

2.4.3. Arsenobetaine assay

The arsenobetaine assay method was modified from Alberti et al. (1995), Dagnac et al. (1999), and Geiszinger et al. (1998). A 200-μl aliquot of the fish muscle extract was injected into HPLC (Hitachi 7100, Naka, Japan) equipped with a cation column (matachem, necleosil, 5 μm, 250×4.6 mm). The mobile phase was 10 mM pyridine that pH was adjusted to 2.9 with formic acid and pump with a flow rate of 1 ml/min. 5% $K_2S_2O_8$ was dissolved in 2.5% NaOH solution (0.8 ml/min) added before UV photooxidation to increase arsenobetaine degradation. Chromatogram of arsenobetaine standard solution and fish muscle was shown in Fig. 1(B1) and (B2). The retention time of arsenobetaine was appearing on about the eleventh minute. The elution order was unknown peak 2, arsenobetaine, and unknown peak 3. The operating condition of arsenic species and total arsenic was shown in Table 1.

2.5. Quality assurance and quality control in the laboratory

The accuracy of procedure was validated by the analysis of standard reference material (SRM) BCR 627 tuna tissue. Total arsenic and DMA concentrations of SRM were 4.9±0.2 mg/kg and 1.8±0.1 μmole/kg in accordance with the certified values of 4.8±0.3 mg/kg and 2.0±0.3 μmole/kg, respectively. The detection limits of total arsenic, As (III), As (V), MMA, DMA, and arsenobetaine were 0.2, 0.4, 0.2, 0.4, 0.3, and 0.5 μg/l correspondingly. Samples were spiked with arsenic species to calculate the recovery rate in every extraction step and laboratory procedure. Extraction recovery rates of As (III), As (V), MMA, DMA, and arsenobetaine were 102.7±4.7%, 104.1±6.8%, 104.7±6.5%, 98.0±7.1%, and 97.7±6.4%, respectively. Laboratory procedure recovery rates of total As, As (III), As (V), MMA, DMA, and arsenobetaine were 103.2±7.1%, 100.7±3.8%, 97.2±4.0%, 104.9±4.6%, 97.2±4.0%, and 97.7±4.7%, respectively. The coefficient of variation (CV) was used to test the reliability and was less than 5% for all experiments.

2.6. Statistical analysis

SAS 8.0 software was used for data analyses. Linear regression was used to analyze the relationship between inorganic arsenic levels in pond water and arsenic species levels in fish. Multivariate analysis was carried out to study the relationship between total arsenic levels in pond water and arsenic species levels of fish adjusted for fish length and weight as covariates.

3. Results

Table 2 showed concentrations of arsenic species (As (III), As (V), MMA, DMA) and total arsenic in 21 aquaculture ponds. Among them, five were located in Putai township, seven in Yichu township, seven in Hsuehchia township, and two in Peiman township. Total arsenic concentration ranged from 4.4μg/l to 302.8 μg/l. In 18 out of 21 ponds, the levels are below 50 μg/l, a safety level specified in the *Drinking Water Act* of Taiwan, 1997. Water samples from Putai-5, Peimen-1, and Peimen-2 had total arsenic concentration well above 50 μg/l. Averaged arsenic levels among ponds were lowest in Hsuehchia (14.4 μg/l), followed by Yichu (15.1 μg/l), Putai (75.8 μg/l), and Peimen (221.0 μg/l), indicating significant geographic differences. However,

despite of the differences in total arsenic concentration, levels of As (III), MMA, and DMA were low or non-detectable, made up only a small percentage of total arsenic concentration, while As (V) is the predominant species, with an exception of Yichu-2 pond, where As (V) is non-detectable.

Mean concentrations of arsenic species and total arsenic in fish collected from the 21 ponds were shown in Table 3. A minimum of three fish were collected from each pond, and tissue samples from 68 fish were analyzed for levels of As (III), As (V), MMA, DMA, arsenobetaine and total arsenic. Results showed that

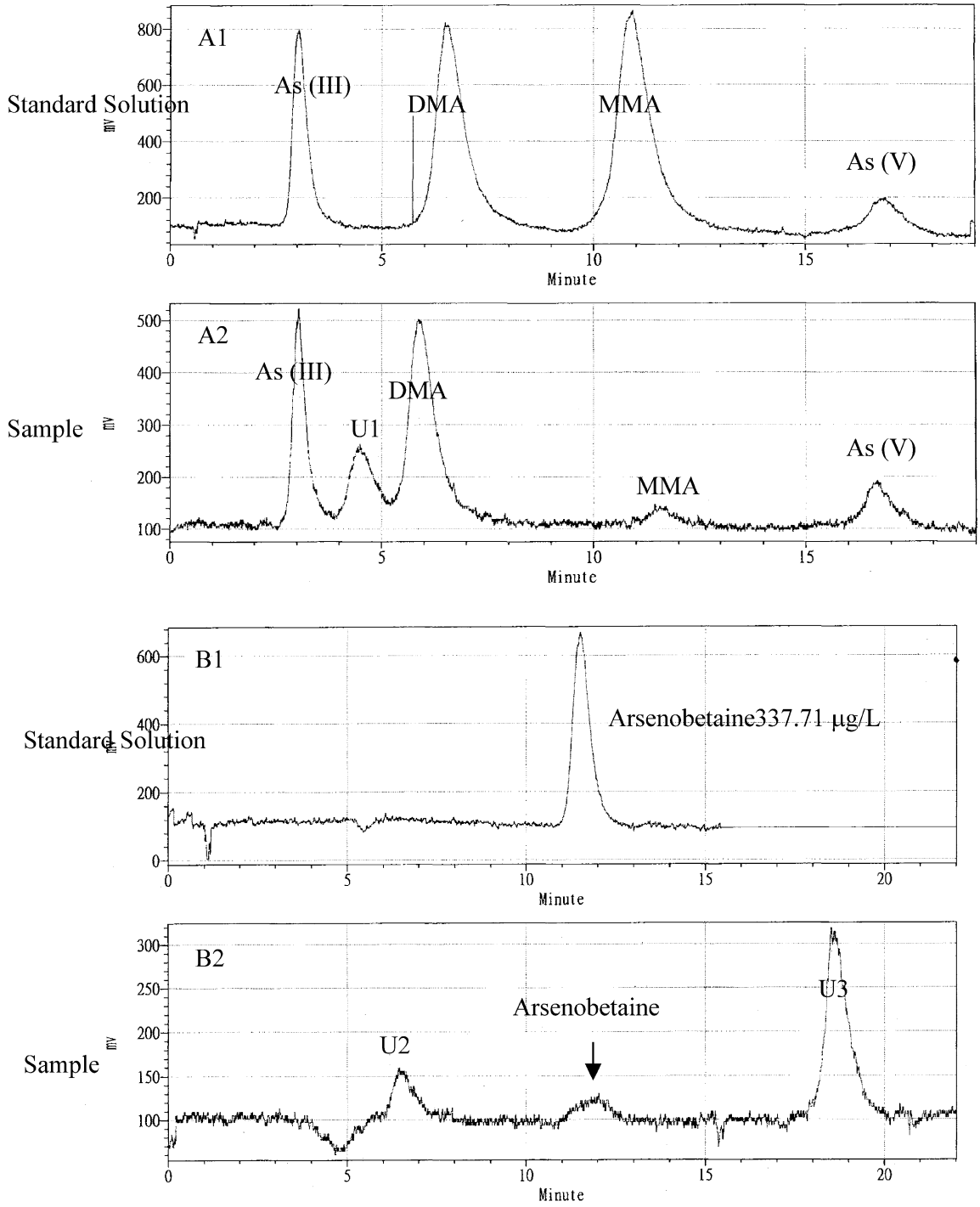

Fig. 1. (A) Inorganic and methyl arsenic species chromatogram: As (III): arsenite, As (V): arsenate, MMA: monomethylarsonic acid, DMA: dimethylarsinic acid; (B) arsenobetaine chromatogram; U1, U2, and U3: unknown species.

their respective range of concentration were 0∼0.3, 0∼0.2, 0∼0.2, 0∼1.5, 0.4×10^{-1}∼2.2, and 0.1∼4.4 μg/g. MMA was not detected in about one third of the fish. On average, As (III), As (V), MMA, DMA, and arsenobetaine made up 3.0, 4.4, 1.9, 20.8, and 58.1% of total arsenic. Arsenobetaine was the major species and MMA was the minor species in fish muscle. Arsenic species and total arsenic levels in fish muscle in Putai were the highest. There were variations in toxicity from arsenic species for biota. Inorganic arsenic species were more toxic. Comparing percentages of inorganic arsenic in fish muscle, Peimen was highest (12.8%), followed by Yichu (8.3%), Hsuehchia (6.4%), and Putai (5.7%). Fish from Putai ponds had the highest total arsenic levels and the lowest inorganic percentage. Inorganic arsenic levels in marine organisms that farmed in arsenic contamination areas are essential to monitor.

Table 1
Operating condition for arsenic species and total arsenic

	Total arsenic	As (III), As (V), DMA, MMA	Arsenobetaine
Hydride system			
Cell temperature	900 °C	900 °C	900 °C
Reducing reagent	0.5% $NaBH_4$ in 0.25% NaOH	0.5% $NaBH_4$ in 0.25% NaOH	0.5% $NaBH_4$ in 0.25% NaOH
HCl	1 N	1 N	1 N
Ar gas	50 psi	50 psi	50 psi
Atomic absorption spectrometry			
Lamp wavelength	193.7 nm	193.7 nm	193.7 nm
Lamp electric current	380 mA EDL system	380 mA EDL system	380 mA EDL system
HPLC			
Buffur	D_2 water	Na_2HPO_4 and NaH_2PO_4	Pyridine
Pump flow	1.5 ml/min	1.5 ml/min	1.0 ml/min
Column	–	Anion ion column	Cation ion column
Degraded reagent	–	–	$K_2S_2O_8$ 5% in 2.5% NaOH 0.8 ml/min
UV light	–	–	54 nm Teflon tube length: 1.14 m

Table 2
Arsenic species and total arsenic concentrations in 21 aquaculture cultures

Arsenic species (μg/l) Culture no. (location)	As (III)	As (V)	MMA	DMA	Total As
Putai-1	ND	23.2	ND	ND	23.9
Putai-2	0.7	36.5	0.3	1	39.4
Putai-3	ND	39.6	ND	ND	44.4
Putai-4	ND	38.5	ND	0.4	45.5
Putai-5	0.1	197	0.4×10^{-1}	ND	209
Putai area Mean±S.E.	0.2±0.1	66.9±32.6	$0.6\times10^{-1}\pm0.5\times10^{-1}$	0.3±0.2	75.8±38.8
Yichu-1	ND	3.5	1.1	0.3	4.6
Yichu-2	ND	ND	0.5	2	6.7
Yichu-3	ND	9.7	ND	ND	11.2
Yichu-4	ND	12	ND	ND	17.5
Yichu-5	0.5	13.6	ND	0.3	20.6
Yichu-6	2.8	15	ND	ND	21.3
Yichu-7	0.5	17.8	0.4	1.9	23.9
Yichu area Mean±S.E.	0.5±0.4	10.2±2.4	0.3±0.2	0.7±0.3	15.1±2.9
Hsuehchia-1	0.4×10^{-1}	4.2	ND	ND	4.4
Hsuehchia-2	ND	5.3	ND	ND	6.7
Hsuehchia-3	ND	4.4	0.3	ND	10.4
Hsuehchia-4	ND	7.1	ND	ND	12
Hsuehchia-5	ND	14	ND	ND	14.3
Hsuehchia-6	ND	12.9	ND	ND	15.9
Hsuehchia-7	ND	30	ND	ND	36.9
Hsuehchia area Mean±S.E.	$0.1\times10^{-1}\pm0.1\times10^{-1}$	11.1±3.5	$0.4\times10^{-1}\pm0.4\times10^{-1}$	ND	14.4±4.1
Peimen-1	ND	60.4	0.5	1.9	64.2
Peimen-2	ND	291.1	ND	8.9	302.8
Peimen mean±S.E.	ND	172.6±118.5	0.2±0.2	5.4±3.5	221.0±138.8
All study areas mean±S.E.	0.2±0.1	39.5±15.5	0.15±0.06	0.8±0.4	48.93±18.4

S.E.: standard error; ND: non detectable.

Table 3
Mean and standard error of arsenic species and total arsenic concentrations in farmed fish

Culture no	No. of fish	Mean ± standard error (μg/g)					
		As (III)	As (V)	MMA	DMA	Arsenobetaine	Total arsenic
Putai-1	3	1.1±0.6	1.2±1.0	4.7±4.0	10.0±3.4	45.1±9.1	67.4±3.0
Putai-2	3	0.4±0.1	2.6±0.6	0.2±0.2	6.3±2.1	24.5±9.5	35.7±11.6
Putai-3	4	3.2±2.4	1.9±0.6	3.8±3.8	19.5±9.6	81.1±11.5	129.6±21.9
Putai-4	3	16.9±6.3	9.2±2.3	1.7±0.8	11.5±18.1	107.5±54.4	329.1±68.7
Putai-5	3	2.5±0.8	0.3±0.2	0.8±0.8	34.0±4.5	169.1±24.5	292.0±66.3
Yichu-1	3	1.2±0.2	2.0±1.0	0.7±0.2	10.0±3.4	34.4±6.3	54.5±7.4
Yichu-2	3	0.9±0.9	1.6±0.7	ND	11.4±4.5	7.8±3.8	21.6±7.6
Yichu-3	3	1.4±0.1	0.3±0.3	1.5±0.1	9.0±2.0	21.9±0.6	40.9±1.8
Yichu-4	3	1.1±0.4	1.5±1.0	1.5±1.5	18.5±8.7	35.1±11.3	66.8±16.4
Yichu-5	3	2.1±1.0	2.2±1.3	ND	3.3±2.2	23.4±2.3	34.9±7.3
Yichu-6	3	2.0±1.1	1.2±0.1	1.3±1.3	10.3±6.8	63.2±4.8	78.8±4.1
Yichu-7	3	3.3±1.3	1.5±0.5	ND	23.1±8.7	8.5±0.9	61.1±22.6
Hsuehchia-1	3	1.0±0.1	0.4±0.2	4.1±4.1	4.6±1.9	25.7±9.8	44.2±0.5
Hsuehchia-2	3	0.6±0.2	1.2±0.8	0.8±0.8	5.9±1.6	18.2±2.5	31.3±0.6
Hsuehchia-3	3	2.0±1.2	5.8±5.0	ND	8.6±2.7	32.3±4.4	50.6±2.0
Hsuehchia-4	7	1.8±0.3	1.4±0.5	0.2±0.2	10.9±3.5	59.9±11.0	91.3±16.2
Hsuehchia-5	3	2.5±1.2	1.2±0.2	ND	7.4±3.3	66.6±14.9	104.8±26.5
Hsuehchia-6	3	4.5±1.9	7.8±2.2	0.9±0.5	25.6±7.5	39.1±1.9	87.8±1.2
Hsuehchia-7	3	0.7±0.6	0.8±0.5	ND	21.7±3.4	30.9±4.5	52.4±6.5
Peimen-1	3	ND	2.3±1.5	ND	5.2±0.8	9.7±2.5	18.4±0.1
Peimen-2	3	7.8±7.6	3.3±0.8	0.2±0.2	6.9±0.9	62.4±14.0	85.9±14.5

Note: all data presented $\times 10^{-2}$.
ND: non detectable.

We found the arsenic levels of fish significantly increased with the arsenic levels of pond water increment. To explore the relationship between arsenic levels of fish and that in ponds water lower than 50 μg/l. Therefore we excluded three ponds which had extremely high total arsenic levels. Fig. 2 shows the relationship between concentrations of inorganic arsenic in water and the concentration of arsenic species in fish muscle. Regression analyses were performed to estimate their relationship. Fig. 2 shows that they were positively related. With the exception of arsenite and MMA, their relationship were statistically significant at 0.05 levels.

Since the concentrations of arsenic species of fish is likely to be affected by the time spent in arsenic contaminated water, the length and weight of fish were used as a proxy measure of time spent in ponds, and were used as control variables. Table 4 shows the results of regression analysis after controlling for weight and length of fish. Three models were presented in Table 4. Model I displays the relationship between arsenic species in fish and total arsenic levels in pond water. The relationship between arsenic species in fish muscle and total arsenic levels of pond water is equivalent to that shown in Fig. 2. Models II and III were adjusted for fish length and weight respectively. Three models show identical relationships, although regression coefficients decreased slightly after adjusting for length and weight. It appears concentration of inorganic arsenic in water is positively related to the concentration of arsenic species in fish.

4. Discussion

Drinking water is the major source of arsenic exposure in humans. Many kinds of food may be contaminated by arsenic such as aquaculture crops during washing and seafood grown in aquaculture. Pumping for irrigation might lead to the release of arsenic into groundwater (Harvey et al., 2002) and affect human health if the groundwater is used for washing or irrigating foodtuffs. Studies have been conducted in Taiwan (Schoof et al., 1998), West Bengal (Roychowdhury et al., 2002), and Mexico (Del Razo et al., 2002) to measure the food arsenic levels in arseniasis areas. The inorganic arsenic concentration of groundwater in arseniasis areas was higher than in other areas (Hsinchu) in Taiwan (Chen et al., 1995b). Arsenic levels of pond water in BFD areas were measured every 2 months from 1998 to 2000, and the average total arsenic concentrations in Putai1, Putai2, Putai3, Yichu1, Yichu2, Hsuehchia1, and Hsuehchia2 ponds were 30.5, 83.4, 169.7, 37.6, 20.2, 79.2, and 47.0 μg/l, respectively (Lin et al., 2001). Total arsenic levels in some of these pond water were higher than the admissible levels stipulated by of the *Drinking Water Act* in Taiwan. Crops growing in the arseniasis areas had higher total arsenic levels than those in other non-arseniasis areas (Schoof et al., 1998). High arsenic levels in pond water possibly result from added groundwater with high arsenic concentrations, or from the pond sediment. The highest prevalence of BFD among those four townships was in

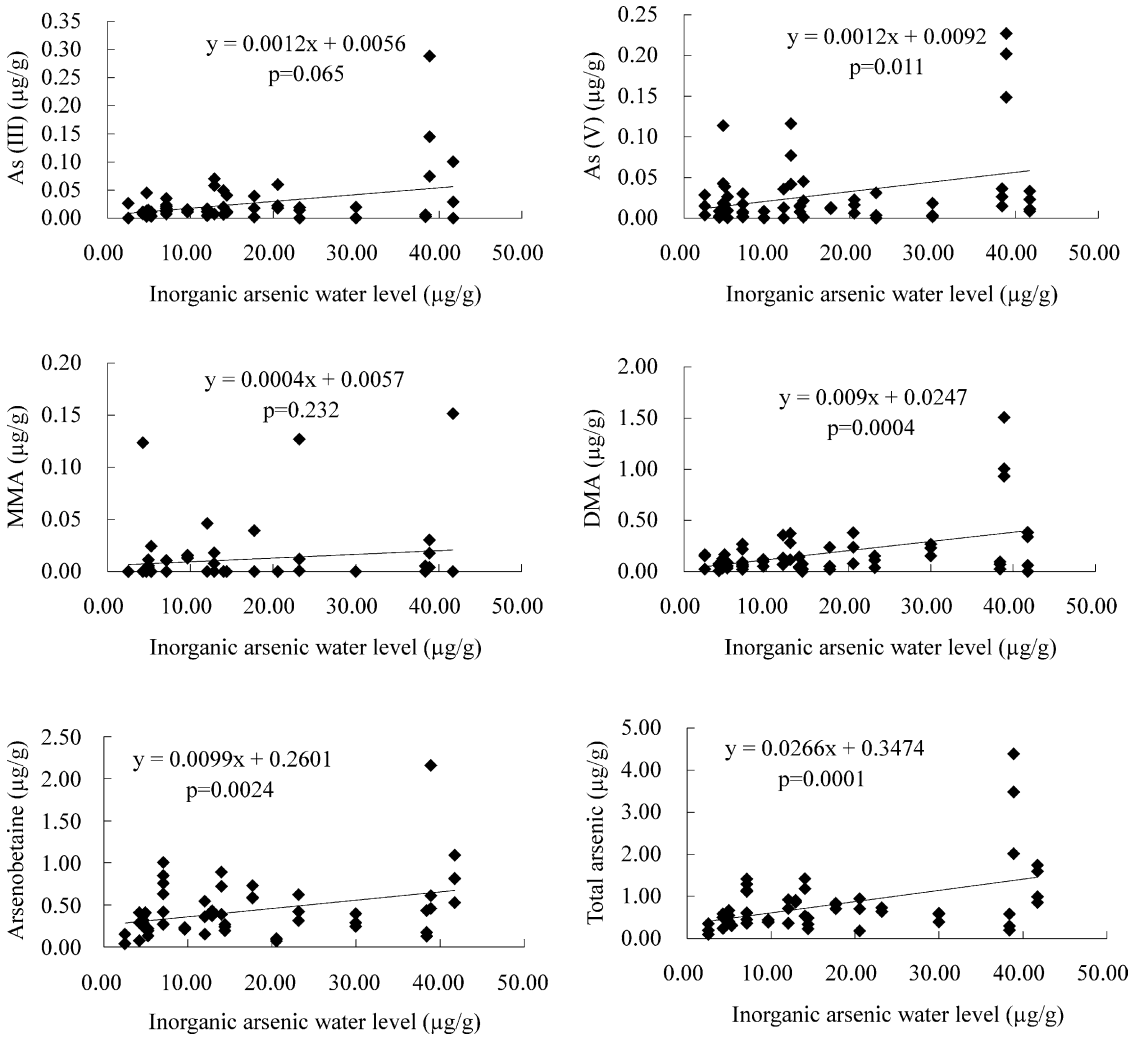

Fig. 2. Scatter plots of arsenic species in fish and inorganic arsenic levels in water.

Table 4
Regression analysis on the level of farmed fish arsenic species and total arsenic level in culture water adjusted for fish length and fish weight

	Model I[a]		Model II[b]		Model III[c]	
	Culture water total arsenic level (μg/l)		Culture water total arsenic level (μg/l)		Culture water total arsenic level (μg/l)	
Fish arsenic species level (μg/g)	β	β (S.E.)	β	β (S.E.)	β	β (S.E.)
Arsenite	1.3**	0.4	1.1*	0.4	1.1**	0.4
Arsenate	1.3**	0.4	1.0*	0.4	1.1*	0.4
MMA	0.2	0.3	0.3	0.3	0.2	0.3
DMA	9.7**	2.2	8.1**	2.2	8.5**	2.3
Arsenobetaine	9.8**	3.2	8.0**	3.2	7.7*	3.1
Total arsenic	27.3**	6.3	22.4**	6.1	23.0**	6.4

Note: β, regression coefficient$\times 10^{-3}$; S.E.: standard error$\times 10^{-3}$.
*$P<0.05$; **$P<0.01$.
[a] Univariate analysis.
[b] Adjusted for fish length.
[c] Adjusted for fish weight.

Peiman (Tseng, 2002), which is consistent with our findings that the pond water in Peiman had the highest total arsenic levels compared to other study areas.

Much research has studied the total arsenic levels in marine and aquatic organisms. Total arsenic levels in different fish species vary from 250 to 3880 g/kg (Sapunar-Postruznik et al., 1996). In our study, total arsenic levels of *O. mossambicus* obtained from dorsal muscle ranged 0.1–1.4 μg/g dry weight, in comparison with other studies using different species of fish for which levels ranged 0.2–2.2 μg/g wet weight (Suner et al., 1999) and 4.1–33.0 μg/g dry weight (Suner et al., 2002). We found that total arsenic levels of farmed fish in arseniasis areas were lower compared to the results of a previous study (flounder, *Psettodes erumei*, 7.9–19.8 μg/g dry weight), where samples were taken from the Putai coast of southwestern Taiwan (Han et al., 1997). The different total arsenic levels between farmed fish and fish from the sea might have resulted from various fish species, analytical methods, and/or sampling strategies in the two studies.

Schoof et al. (1999) reported that As (III) and As (V) concentrations in saltwater finfish (cod, halibut, orange roughy, and canned fish) were less than 1 ng/g (Schoof et al., 1999) and Munoz et al. (2000) also reported that the levels of inorganic arsenic in white fish were very low (0.01–0.1 μg/g). In contrast, the range of inorganic arsenic levels of *O. mossambicus* in our study was greater than 0.3 μg/g (dry weight). Also, pond water was reported to have higher arsenic levels than seawater (1.8 μg/l) (Braman et al., 1973) and coastal water (0.9 μg/l) (Valette-Silver et al., 1999). The above findings suggest that environmental arsenic levels greatly affect inorganic arsenic concentrations of organisms. Therefore, the inorganic arsenic levels in aquaculture fish in this study were higher than those of saltwater fish.

A laboratory study was conducted to investigate the bioaccumulation and biotransformation of different arsenic species in *Tilapia mossambica* cultured in fresh water, with different arsenic species and levels (Suhendrayatna et al., 2002b). The authors found that *T. mossambica* transformed arsenite to arsenate via the freshwater food chain (Suhendrayatna et al., 2002b). Thus, we thought that even though *O. mossambicus* was exposed to arsenite in the pond water, it might be transformed to arsenate. This might be the reason that we found no significant correlation between arsenite levels of fish and inorganic arsenic levels of pond water. The mechanism of arsenite oxidization in fish differs from that of human metabolic processes, by which arsenate is reduced to arsenite (Radabaugh et al., 2000).

Methyl arsenic species were found in inorganic arsenic-exposed marine organisms (Suhendrayatna et al., 2002a; Suhendrayatna et al., 2002b). This and many other previous studies (Munoz et al., 2000; Suhendrayatna et al., 2002a,b; Suner et al., 2002; Velez et al., 1996) found that the percentage of MMA was very low in marine organisms. We were unable to detect MMA in about one-third of our samples. We also found that DMA levels and percentages of DMA in our study were higher than those reported by other studies (Munoz et al., 2000; Suhendrayatna et al., 2002a,b; Suner et al., 2002; Velez et al., 1996), while arsenobetaine concentrations and percentages of arsenobetaine were lower than those of other studies (Suhendrayatna et al., 2002a,b; Suner et al., 2002). One reason might be that arsenobetaine was degraded to DMA by fish intestinal microbes (Hanaoka et al., 1992). Or, arsenobetaine might have low bioaccumulation in fish via the food chain in our study area, because different species of algae or microbes may have different synthesis abilities (Cullen et al., 1989).

We found that fish size had a significant relationship with arsenic levels. However, only a few of studies have mentioned this phenomenon. Inorganic arsenic methylation also varies in different marine species. After adjusting for fish growth factors (length or weight) to account for different times spent in arsenic-contaminated water, a significant relationship still existed between arsenic concentrations in pond water and in fish. This further supports our conclusion that arsenic levels in *O. mossambicus* are influenced by arsenic levels in the aquaculture environment.

Arsenic originates from various sources in the environment. In addition to drinking water, arsenic food contamination may also cause adverse health effects. In this study, we found that inorganic arsenic levels in fish were dependent on total arsenic concentrations of pond water. Inorganic arsenic species are more toxic than methyl arsenic species. Therefore the effect of inorganic arsenic species might result in a greater number of adverse health effects to the general public. Determining the percentages of arsenic species in marine organisms may better characterize the human heath effects caused by arsenic exposure from seafood consumption. It is of importance to evaluate the inorganic arsenic levels of farmed seafood in arsenic-contaminated areas.

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