Spatiotemporal distribution of arsineic species of oysters (*Crassostrea gigas*) in the coastal area of southwestern Taiwan

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Received: 7 September 2006 / Accepted: 11 April 2007 / Published online: 15 May 2007 © Springer Science + Business Media B.V. 2007

Abstract This study investigated total arsenic (As) and As species contents of oysters (*Crassostrea gigas*) in different production areas, seasons and sea locations on the southwestern coast of Taiwan. Analytical results indicate that contents of total As, arsenite, arsenate, dimethylarsinic acid, monomethylarsonic acid and arsenobetaine in oysters are $9.90\pm$ 3.68, 0.091 ± 0.104 , 0.033 ± 0.038 , 0.529 ± 0.284 ,

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C.-S. Jang Department of Leisure and Recreation Management, Kainan University, Luzhu, Taoyuan, Taiwan 33857, Republic of China 0.037±0.046 and 3.94±1.33 mg/g (dry wt), respectively. A ratio of inorganic As concentrations to total As concentrations is 1.26%. Total As contents of oysters cultured in the outer sea are statistically significantly lower than those of oysters cultured in the inner sea. The total As contents of oysters is the highest in Putai, where the blackfoot disease prevails. The low As contents in oysters is attributed to the low temperature in winter, which slows the metabolism of oysters. A maximum value is 33.37 µg/g (dry) in Putai in spring, because a considerable amount of aquacultural waste water with high As contents is discharged into adjacent drainage channels and rivers there during that season.

Keywords Oyster · Arsenic species · Blackfoot disease · Spatiotemporal distribution

Introduction

Oysters *(Crassostrea gigas)* are one of the most favored edible shellfish in Taiwan and their production value is the highest of all cultured shellfishes. Most oysters are cultured in coastal areas of southwestern Taiwan. Blackfoot disease (BFD) has been prevailed in these regions. Many epidemiological studies have shown that drinking groundwater with a high arsenic (As) content is strongly related to the BFD occurrence (Ch'i and Blackwell 1968). Nowadays, most inhabitants in the regions do not drink well water directly, but groundwater is still extensively utilized to farm fish and shellfish (Liao and Ling 2003). Arsenic contents in surface water normally range from 0.15 to 5 μ g/l. The Taiwan EPA (2002) surveyed the quality of water in six subordinate rivers in western Taiwan from December 2001 to April 2002. An As concentration of 16 μ g/l was detected in the Jiangjun river (see Fig. 1), which is located in the BFD area. Lin (2004) also collected water samples from nearby rivers. The analytical results reveal that the As concentrations in the neighboring rivers range from 13 to 45 μ g/l. The bioaccumulation of high As concentration from river water to the farmed oyster in the BFD coastal area is of great concern.

Oysters in Taiwan are generally farmed artificially. Farmed types in the shallow inner sea are classified as horizontal or vertical types of racks, based on the geographical, seasonal and tidal conditions (Lin and Liang 1982). These types of oysters are exposed to air for some of the day, because of the cyclic ocean tide. Additionally, the aquacultural type of racks in the deep outer sea has been developed to prevent pollution of the oyster by river water. This aquacultural



Fig. 1 Locations of oyster sampling. The gray region represents the BFD hyperendemic areas

type is located far from the coast and is therefore less affected by the ocean tide than other farmed types of oysters. Oysters living in the outer sea grow faster than those in the inner coastal sea. Accordingly, the bioaccumulation of As species in oysters using different culturing types must be examined.

Organic As usually predominates in marine organisms. Inorganic As is more toxic and carcinogenic than organic As (Oremland and Stolz 2003). Consequently, the inorganic As content in food, rather than the total As, is used to assess the carcinogenic risks (U.S. EPA 1998; 2004). However, the inorganic As content in oysters and its seasonal variation are unavailable in Taiwan. The assessed carcinogenic risks associated with inorganic As contents in oysters by assuming a particular percentage of total As contents involve considerable levels of uncertainty.

This work conducted a 12-month survey of As species contents of oysters in the main oyster production areas of Taiwan, including total As, arsenite (As(III)), arsenate (As(V)), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and arsenobetaine (AsB), to establish the spatiotemporal variation of total As and As species contents in oysters in the southwestern coast of Taiwan. The measured concentrations of total As and As species in oysters were statistically analyzed for various production areas, seasons and sea locations. The spatial/seasonal distributions of total As and As species of oysters were evaluated to determine pollution sources of As. The analyzed result provides an important reference to government for controlling pollution and for assessing risks from exposure of inorganic As through oyster consumption in Taiwan.

Materials and methods

Sample collection and analysis

Oyster samples were collected from southwestern offshore aquaculture farms in Wangkung, Tungshih, Putai and Anpin. The Putai was one of the BFD regions (Fig. 1). Oyster samples were purchased monthly at two sites in each township and three oyster samples from each site were analyzed to determine their total As and As species contents. However, no purchase was made in January and February in Wangkung; in February and March in Putai; and in January, February and April in Tungshiu. The total number of oyster samples was 252. The average size of the oyster is around 4 cm in diameter with a wet weight of 10 g. Oyster samples were frozen while they were transported from the field to the laboratory and stored at -20° C until they were dissected.

A portion of the homogenized samples were freeze–dried for 36 h and prepared to analyze total As contents and particular As species contents. About 0.5 g of homogenized freeze–dried samples and 25 ml of 65% nitric acid were added to a flask. They were boiled and decanted to allow gases to pass through a condenser. They were digested for 12 h until the solution was clear. Total As contents were analyzed using an electro-thermal atomic absorption spectrometer (AAS) (AA100 Perkin-Elmer Shelton, USA), and a hydride generator (HG) (FIAS 400 Perkin-Elmer Shelton, USA). 0.5% NaBH4 in 0.25% NaOH and 1 N HCl were added into 200 μ l of a digested sample to reduce the arsenic to arsine. The total arsenic concentrations were determined by HG/AAS.

Freeze-dried oysters of $1.0 \sim 1.5$ g and 150 ml of methanol/water solution (1/1, v/v) were placed into a Soxhlet extraction apparatus, modified from that described by Gomez-Ariza et al. (2000a) and extracted for 16 h. A methanol extraction tube was designed to remove the methanol when the extraction was completed. After the methanol was removed, the extract was freeze-dried to a powder and re-dissolved in 10 ml of de-ionized water. The re-dissolved liquids were purified by filtering through C18 cartridges. A high-

Table 1 Operating condition for As species and total As

performance liquid chromatograph, HPLC (Hitachi 7110, Naka, Japan), equipped with an anion column (Machey-Nagel, Nucleosil, 10 µm, 250×4.6 mm) and connected to HG/AAS, was used to separate As (III), As (V), MMA and DMA. The procedures for analyzing As (III), As(V), MMA and DMA followed closely those in our earlier work (Huang et al. 2003). The AsB assay method was modified from Alberti et al. (1995), Dagnac et al. (1999), and Geiszinger et al. (1998). A 200-µ aliquot of the fish muscle extract was injected into HPLC (Hitachi 7100, Naka, Japan) equipped with a cation column (matachem, necleosil, 5 μ m, 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. Five percent K2S2O8 was dissolved in 2.5% NaOH solution (0.8 ml/min) added before UV photooxidation to increase AsB degradation. Table 1 summarizes the operating conditions for analyzing total As contents and the contents of particular As species.

The accuracy of the procedure was verified by the analysis of the standard reference material (SRM) BCR 627 tuna tissue. Total As and DMA concentrations of SRM were 4.9 0.2 and 1.8 0.1 μ mole/kg, respectively, which values were consistent with the respective certified values of 4.8 0.3 mg/kg and 2.0 0.3 μ mole/kg. The detection limits of total As, As (III), As (V), MMA, DMA and AsB were 0.2, 0.4, 0.2, 0.4, 0.3 and 0.49 μ g/1, respectively. Samples were spiked with As species to calculate the recovery rate in every extraction step and laboratory procedure.

	Total AS	As(III), As(V), DMA, MMA	AsB
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
HC1	1N	1N	1N
Ar gas	50 psi	50 psi	50 psi
Atomic absorption spect	trometry		
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			
Buffer	D ₂ water	Na ₂ HPO ₄ and NaH ₂ PO 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 ₂ S ₂ O ₈ 5% in 2.5% NaOH 0.8 ml/min
UV light	_	_	54 nm Teflon tube length: 1.14 m

The extraction recovery rates of As (III), As (V), MMA, DMA and AsB were 102.7 4.7, 104.1 6.8, 104.7 6.5, 98.0 7.1 and 97.7 6.4%, respectively. The laboratory procedure recovery rates of total As, As (III), As (V), MMA, DMA and AsB were 103.2 7.1, 100.7 3.8, 97.2 4.0, 104.9 4.6, 97.2 4.0 and 97.9 4.7%, respectively. The coefficient of variation was used to test the reliability and was less than 5% for all experiments.

Statistical analysis

The oyster samples can be grouped by three factors – area, season and sea location. Analysis-of-variance (ANOVA) was thus applied to examine the statistical differences of the dependent variables - the total As contents and the contents of particular As species in oysters as they varied with each factor. The seasonal factor was specified as one of four levels - spring (March to May), summer (June to August), fall (September to November) and winter (December to February); the area factor also had four levels -Anpai, Putai, Tungshih and Wangkung, whereas the sea location had two levels - inner sea and outer sea. SPSS 10.0 software was used for statistical analyses. A 0.05 level of significance (α =0.05) was used to test whether the mean concentrations of total As and particular As species in oysters differ among the groups. When the differences among the dependent variables were statistically significant, multiple comparisons were made using the post hoc test by the Scheffe method (Norusis 1998) to distinguish the paired groups when the factors had more than two levels.

Results

Table 2 lists the monthly measured total As concentrations in oysters collected from the four townships. The highest and the lowest total As concentrations in the four townships are $22.90\pm7.41 \ \mu g/g \ (mean\pm SD)$ in Putai in April and 4.22 ± 0.32 µg/g in Anpin in February. The seasonal concentrations of total As in oysters are 9.96±4.92, 9.94±2.67, 10.75±3.42 and 8.52 ± 2.75 µg/g in spring, summer, fall and winter, respectively (Table 3). The total As concentrations in oysters are 8.39±2.25, 11.12±5.20, 9.62±2.39 and 10.30 ± 3.15 µg/g in Anpin, Putai, Tungshih and Wangkung, respectively (Table 4). Putai has the highest mean total As concentration, whereas Anpin has the lowest. The inorganic As fractions in spring, summer, fall and winter were 1.33, 1.21, 0.82 and 1.88%, respectively (Table 3), while the inorganic As fractions in Anpin, Putai, Tungshih and Wangkung are 1.21, 1.51, 1.28 and 1.02%, respectively (Table 4).

For the seasonal variation (Table 3), the total As content of oysters in fall significantly exceeds that in winter (p<0.01); the As(III) content of oysters in spring significantly exceeds that in fall (p<0.05); the As(V) content of oysters in winter significantly exceeds that in fall (p<0.01); and the DMA content

Month	Total As concent	Total As concentration (mean±SD)					
	Anpin	Putai	Tungshih	Wangkung	4 townships average		
January	12.39±1.25	11.66±0.60	_	_	11.90±0.86		
February	4.22 ± 0.32	_	_	-	4.22 ± 0.32		
March	$7.60 {\pm} 0.73$	-	6.05 ± 0.45	7.55±1.26	7.20 ± 1.05		
April	$8.00 {\pm} 0.50$	22.90 ± 7.41	_	$7.80{\pm}2.05$	12.97 ± 8.35		
May	$8.45 {\pm} 0.77$	9.85±0.74	10.44 ± 1.00	13.14 ± 0.23	10.47 ± 1.87		
June	$6.39 {\pm} 0.73$	11.64 ± 3.92	12.25 ± 0.18	10.78 ± 2.67	10.27 ± 3.25		
July	11.53 ± 2.49	$7.69 {\pm} 0.54$	$9.44 {\pm} 0.92$	8.17±0.55	9.21±1.99		
August	9.80±0.21	12.71 ± 4.18	11.11 ± 0.93	9.65±1.68	10.96 ± 2.63		
September	$10.07 {\pm} 0.26$	13.33 ± 3.40	7.52±1.13	12.25 ± 2.36	10.90 ± 3.23		
October	12.16 ± 4.05	9.42±0.17	11.55 ± 3.02	16.36±2.29	12.39 ± 3.61		
November	8.43 ± 2.74	11.42 ± 3.44	8.28 ± 3.28	8.01 ± 0.98	9.10±3.01		
December	$7.65 {\pm} 0.46$	7.21 ± 2.86	9.73 ± 0.36	$9.18 {\pm} 0.63$	$8.03 {\pm} 2.27$		

Table 2 Monthly measured total As concentrations ($\mu g/g$) in oysters from Anpin, Putai, Tungshih and Wangkung

As species	As species concentration (mean±SD (μ g/g))				
	Spring (69) ^a	Summer (66)	Fall (69)	Winter (48)	
As(III)	0.118±0.174*	0.084 ± 0.049	0.064±0.054*	0.102 ± 0.062	
As(V)	$0.033 {\pm} 0.045$	$0.034{\pm}0.027$	0.023±0.024**	0.049±0.050**	
DMA	0.501 ± 0.420	0.621±0.201*	0.473±0.197*	0.521±0.211	
MMA	$0.038 {\pm} 0.054$	$0.048 {\pm} 0.045$	$0.030 {\pm} 0.035$	$0.033 {\pm} 0.047$	
AsB	3.96±1.27(45)	d	_	3.91±1.46(27)	
Total As	9.96±4.92	9.94±2.67	10.75±3.42**	8.52±2.75**	
Inorganic As ^b /Total As (%)	1.33 ± 0.84	1.21 ± 0.62	$0.82{\pm}0.58$	1.88 ± 1.04	
Organic As ^c /Total As (%)	55.98±21.21	_	_	57.37±12.01	

Table 3 Seasonal distributions of total As and As species in oysters

^a The number inside the parenthesis denotes the sample number.

^b Inorganic As is the summation of As(III) and As(V).

^c Organic As is the summation of DMA, MMA and AsB

^d No data

*p value>0.05

**p value>0.01

of oysters in summer significantly exceed that in winter (p < 0.05). The MMA and AsB contents of oysters, however, do not vary significant among seasons. For the spatial variation (Table 4), the total As contents of oysters in Putai and Wangkung significantly exceed that in Anpin (p < 0.01 and p < 0.05, respectively); the As(III) content of oysters in Putai significantly exceeds those in the remaining

areas (at least p < 0.05); the DMA content of oysters in Anpin is significantly lower than those in the remaining areas (p < 0.01); the AsB content of oysters in Anpin significantly exceeds that in Putai (p < 0.01); but the As(V) and MMA contents of oysters do not vary significantly among the areas. Figure 2 shows spatiotemporal variations of total As and As species contents in oysters (interaction variations of seasons

Table 4 Spatial distributions of total As and As species in oysters

Arsenic species	As species concentration (mean±SD (μ g/g))					
	Anpin (63) ^a	Putai (72)	Tungshih (57)	Wangkung (60)	Four township average	
As(III)	$0.068 {\pm} 0.055^{**}$	0.131±0.169 ^{**, *(1), *(2)}	$0.081 {\pm} 0.054^{*(1)}$	$0.078 \pm 0.052^{*(2)}$	0.091±0.104	
As(V)	0.031 ± 0.039	$0.039 {\pm} 0.041$	$0.038 {\pm} 0.039$	$0.025 {\pm} 0.031$	$0.033 {\pm} 0.038$	
DMA	$0.323 \pm 0.185^{**(1), **(2), **(3)}$	$0.606 {\pm} 0.375^{**(1)}$	$0.582{\pm}0.184^{**(2)}$	$0.599{\pm}0.212^{**(3)}$	$0.529 {\pm} 0.284$	
MMA	0.028 ± 0.033	$0.043 {\pm} 0.057$	$0.039 {\pm} 0.048$	$0.039 {\pm} 0.039$	$0.037 {\pm} 0.046$	
AsB	4.47±1.28 ^{** (27)}	3.29±1.48 ^{** (24)}	3.65±0.79(9)	4.29±0.88 (12)	3.94±1.33	
Total As	8.39±2.25 ^{**, *}	11.12±5.20**	9.62±2.39	$10.30 \pm 3.15^*$	9.90 ± 3.68	
Inorganic As ^b / Total As (%)	1.21 ± 0.92	1.51±0.92	1.28 ± 0.78	1.02 ± 0.65	1.26 ± 0.85	
Organic As ^c / Total As (%)	62.29±14.72	44.37±19.00	59.75±7.37	65.32±18.40	56.50±18.22	

^a The number inside the parenthesis denotes the sample number.

^b Inorganic As is the summation of As(III) and As(V).

^c Organic As is the summation of DMA, MMA and AsB

* p value>0.05

** p value>0.01

*(), **(): Different paired comparisons



Fig. 2 Box-and-whisker plots of the spatiotemporal distributions of \mathbf{a} As(III), \mathbf{b} As(V), \mathbf{c} DMA, \mathbf{d} MMA, \mathbf{e} AsB and \mathbf{f} total As in oysters

and areas) using box-and-whiskers plots. Because of a complicated relationship among the data, a detailed comparison of the data is not reported in this work. Notably, the contents of total As, As(III) and DMA of oysters in Putai in spring are distributed widely and

significantly higher than those in the other areas in the other seasons.

Additionally, this study analyzed the variation at the different sea locations – inner sea and outer sea in which oysters are cultured. However, only the total As content of oysters varies significantly with the interaction between the season and sea location, and the interaction between the area and the sea locations (p<0.01). Figure 3a and b plot the total As content of oysters for various seasons and sea locations, and various areas and sea locations, respectively. The total As content of oysters in the inner sea typically exceeds that in the outer sea.

Discussion

Sanchez-Rodas et al. (2002) analyzed the same species of oysters (C. gigas) farmed off the Atlantic coast of Spain and showed that the total As concentration of oysters was 17.24 ± 0.25 µg/g. Vilano and Rubio (2001) also analyzed the oysters farmed in northwest Spain. They found that a total As concentration in the oysters was 9.74 ± 0.37 µg/g. Kohlmeyer et al. (2002) examined the oysters farmed in the Arcachon bay of France and measured a total As concentration of 26.7 \pm 0.5 µg/g. The measured total As concentrations of oysters in the previously cited studies varied less and all exceeded the value herein of 9.90 ± 3.68 µg/g. In this work, the total As concentrations in oysters vary significantly among the four townships. A spatial variation of total As concentrations of oysters is evident. Additionally, Edmonds and Francesconi (1993) reported that 1.4% of the total arsenic in oysters (C. gigas) from Japan was inorganic As. Kohlmeyer et al. (2002) found that 3% of the total arsenic in the oysters from the northwest of Spain was inorganic. The mean inorganic As content of 1.26% herein is lower than those obtained for Japan and Spain.

This work shows that the total As contents of oysters in Putai ranges from 3.11 to 33.37 μ g/g, with a mean of 11.12 μ g/g (a geometric mean of 10.11 μ g/g). The highest value of 33.37 μ g/g (dry) is found in spring (Fig. 2f). Han et al. (1997) reported the mean concentration of total As in oysters is 18.7 μ g/g (dry) in Putai with range of 12.3~21.4 µg/g (dry). Meanwhile, Han et al. (2000) also reported that the mean concentration of total As in oysters is $4.86 \,\mu g/g$ (dry) in Putai with range of $3.15 \sim 7.0 \ \mu g/g$ (dry). The difference of the total As contents of oysters between the two surveys was a factor of approximately four. Gomez-Ariza et al. (2000a) and Sanchez-Rodas et al. (2002) obtained similar results -3.2 and $18.4 \mu g/g$ (dry), respectively, in the Atlantic southwestern coast in Spain. Thus, the total As content in oysters may widely vary with sampling time at a particular site. A likely reason is that the total As concentrations in oysters vary seasonally.

Most aquaculture in inner sea is conducted in sandbanks and lagoons. Neither the tide nor river pollution affects this aquaculture. Thus, oysters farmed by inner sea aquaculture may ingest the polluted sediments near inner sea. In this study, the total As content in oysters cultured in the outer sea is significantly lower than that cultured in the inner sea (p<0.01, Fig. 3a and b). Gomez-Ariza et al. (2000a) analyzed the As species contents in oysters and sediments in the Tinto and Odiel rivers and the Guadiana river mouth in Spain.



Fig. 3 Box-and-whisker plots of a seasonal and b spatial distributions of total As contents of oysters in the inner and outer seas

Their analytical results indicated that the As(V) contents in the sediments and oysters in the Tinto and Odiel rivers were 10 times higher than those in the Guadiana river mouth. Consequently, the total As content in sediments is closely correlated with that in oysters. Furthermore, Sanchez-Rodas et al. (2002) stated that the total As content in oysters living inside the bay exceeded that in oysters living outside the bay.

Arsenic in oysters is bio-accumulated from environments and by ingestion. The total As content in oysters depends on the sea environments in which it lives. The As(III) content of oysters in Putai significantly exceeds those in the remaining areas and the As(V) content of oysters in Putai slightly exceeds those in the remaining areas (without statistical significance). Blackfoot disease has been prevalent in Putai. Nowadays, groundwater with a high As content in this region is used for aquaculture and washing. Generally, aquacultural ponds that have been used for a few years are emptied of water, causing that pond sediments are exposed to air and sunshine to improve their quality. Large amounts of aquacultural and washed waste water with high As contents are discharged into adjacent drainage channels and rivers. The Taiwan EPA (2002) has monitored the quality of water in the Jiangjun river, located in the vicinity of the BFD, from December 2001 to April 2002. Total As concentrations of 119 μ g/g (wet) and 59 μ g/g (wet) were found in the river sediment, while the total As concentrations of 19 and 16 µg/l were detected in the river water. These data indicate that the As contents of rivers and sediments adjacent to the BFD area exceed those in other areas. The high As contents in the rivers and sediments in the BFD area increase the As content of the neighboring ocean.

Aquacultural ponds in lands are typically exposed to air from November to March. Therefore, oysters cultured in spring are generally exposed to high As environments. In this work, the total As content of oysters in Putai in spring averages 16.38 μ g/g (dry), ranging from 9.0 to 33.37 μ g/g (dry). Total As contents measured in the vicinal channels of the BFD regions are typically high (Huang et al. 2003; Lin 2004). Furthermore, data on the quality of river water monitored by the Taiwan EPA (2002) show that marine-living organisms may bio-accumulate As from the river water with a high As content, from drainage

in the coastal area of southwestern Taiwan. Wilson et al. (1992) found that the total As contents of oysters (*Crassostrea virginica*) from coastal waters of the U.S. Gulf of Mexico contain $4.1-39 \mu g/g$. Their results are close to the total As contents of oysters in Putai measured during spring. Furthermore, the As concentrations were the highest in oysters from the coastal areas along the west coast of Florida in the USA, which receive drainage from natural phosphate mineral deposits, which are rich in As. Arsenic-contaminated drainage from the land may be an important source of As taken up by farmed oysters.

The order of seasonal variation of the total As content in oysters is fall > spring summer > winter. The total As contents of oysters in fall is the highest, revealing high total As contents in the all areas in fall (Fig. 2). Meanwhile, the total As contents are high only in Putai in spring and are considerably low in the remaining areas in spring. Although concentrations of metals in bivalve molluscus are known to vary markedly with the reproductive cycle and the relative development of gonadic tissue, two causes may explain low total As contents of oysters in winter. The first is that Wangkung and Tungshih do not produce oysters in winter, because of the northeasterly winds and ocean current. The oyster samples in winter were taken only from Putai and Anpin. The total As contents of oysters in Putai and Anpin during winter are lower than those in the remaining areas in the other seasons. Therefore, the mean total As content of oysters is lower in winter. The other cause is that the sea is colder in winter. The Taiwan EPA has monitored the quality of water every month in the western Taiwan ocean from October 1997 to November 2003. The mean temperatures of sea water in Tungshih are 25, 30, 26 and 20°C in spring, summer, fall and winter, respectively. The mean temperatures of sea water in Putai are 25, 29, 26 and 20°C in spring, summer, fall and winter, respectively (Taiwan EPA 2002). The monitored data reveal that the temperature in winter is 50°C below those in the other seasons. Denton and Burdon-Jones (1981) showed that the increasing water temperature increases the capacity of shellfish absorbing heavy metals. The amount eaten by oysters and the activity of oysters (C. virginica) are high when the water temperature exceeds 25°C and falls as the water temperature decreases (Hung 1982). Valette-Silver et al. (1999) observed the bioaccumulation of As in

oysters. It varied with the seasonal changes in temperatures and saline concentrations, and with the type of sediments.

The DMA contents are greater than the As (III) and MMA contents in oysters. The analyzed result is similar to those obtained by Gomez-Ariza et al. (2000a, b) and Kirby et al. (2002). In a methylation process of organisms, As(V) contents first transform to As(III) contents, and then transform to MMA and DMA contents (Shiomi et al. 1996). The DMA and MMA contents in organisms are correlated with their metabolism. High DMA and MMA contents in organisms indicate a good capacity of the metabolism. Furthermore, a mean ratio of organic As contents to total As contents in clams is around 56.5% in this study. The main organic As species is AsB. Oysters are a filter-feeding bivalve species and ingest planktons and organic materials. The AsB contents in oysters obtain directly by ingesting those in their food and transform from inorganic As (Cullen and Reimer 1989). According to our analyzed results of the spatial variation of total As and As species (Table 4), AsB contents in oysters are negatively correlated with total As contents in oysters. The DMA contents in oysters copiously appear in Patui, revealing a good capacity of metabolism of oysters in this region. Thus, oysters ingesting food with AsB contents are a likely reason that AsB contents differ at various areas.

Conclusions

This work presented spatiotemporal distributions of total As and As species in oysters in four coastal areas of southwestern Taiwan. The total As contents in oysters in the regions are 9.90 ± 3.68 µg/g (dry wt) during 2002. For the spatial variation, the total As, As (III) and DMA contents of oysters are the highest in Putai, but the AsB contents are lowest in Putai. In the BFD regions, groundwater with high As contents significantly enhances the As exposure to oysters and the As accumulation in oysters. For the seasonal variation, the total As contents of oysters are the highest in fall; the As(III) contents of oysters are the highest in spring; the As(V) contents of oysters are the highest in winter; and the DMA contents of oysters are the highest in summer. The low As contents in oysters in winter is attributed to the low

temperature slowing the metabolism of oysters. For the spatiotemporal variation, the total As, As(III) and DMA contents of oysters are the highest in Putai in spring. Furthermore, the total As contents of ovsters living in inner sea spatiotemporally exceed those living in outer sea. The ratios of inorganic As concentrations to total As concentrations spatiotemporally range from 0.82 to 1.88%, with a mean of 1.26%. Additionally, this study analyzes organic As contents which are approximately 56.5% of total As contents. Main organic As species is AsB. The AsB content in oysters is correlated with their ingestion in habitants. This analyzed result can provide an important reference to government for allocating farmed oysters from inner sea to outer sea, especially in one of the BFD regions, Putai, where discharge waste water contains high As; for characterizing seasonal cycles of As bioaccumulation in oysters; but also for assessing risks from exposure of inorganic As through oyster consumption in Taiwan

Acknowledgements The authors would like to thank the National Science Council of the Republic of China for financially supporting this research under Contracts Nos. NSC90-2313-B-002-322 and NSC 91-2313-B-002-270.

References

- Alberti, J., Rubio, R., & Rauret, G. (1995). Extraction method for arsenic speciation in marine organisms. *Fresenius Journal of Analytical Chemistry*, 351, 420–425.
- Ch'i, I. C., & Blackwell, R. Q. (1968). A controlled retrospective study of blackfoot disease, an endemic peripheral gangrene disease in Taiwan. *American Journal of Epidemiology*, 88, 7–24.
- Cullen, W. R., & Reimer, K. J. (1989). Arsenic speciation in the environment. *Chemical Reviews*, 89, 713–764.
- Dagnac, T., Padro, A., Rubio, R., & Rauret, G. (1999). Speciation of arsenic in mussels by the coupled system liquid chromatography UV irradiation hydride generation inductively coupled plasma mass spectrometry. *Talanta*, 48, 763–772.
- Denton, G. R. M., & Burdon-Jones, C. (1981). Influence of temperature and salinity on the uptake, distribution and depuration of mercury, cadmium and lead by the Black-lip oyster Saccostrea echinata. *Marine Biology*, 64, 317–326.
- Edmonds, J. S., & Francesconi, K. A. (1993). Arsenic in seafoods: Human health aspects and regulations. *Marine Pollution Bulletin*, 26, 665–674.
- Geiszinger, A., Goessler, W., Kuehnelt, D., Francesconi, K., Kosmus, W. (1998). Determination of arsenic compounds in earthworms. *Environmental Science & Technology*, 32, 2238–2243.

- Gomez-Ariza, J. L., Sanchez-Rodas, D., Giraldez, I., & Morales, E. (2000a). Comparison of biota sample pretreatments for arsenic speciation with coupled HPLC-HG-ICP-MS. *Analyst*, 125, 401–407.
- Gomez-Ariza, J. L., Sanchez-Rodas, D., Giraldez, I., & Morales, E. (2000b) A comparison between ICP-MS and AFS detection for arsenic speciation in environmental samples. *Talanta*, 51, 257–268.
- Han, B. C., Jeng, W. L., Hung, T. C., Ling, Y. C., Shieh, M. J., & Chien, L. C. (2000). Estimation of metal and organochlorine pesticide exposures and potential health threat by consumption of oysters in Taiwan. *Environmental Pollution*, 109, 147–156.
- Han, B. C., Jeng, W. L., Jeng, M. S., Kao, L. T., Meng, P. J.,& Huang, Y. L. (1997). Rock-shells (Thais clavigera) as an indicator of As, Cu, and Zn contamination on the Putai coast of the black-foot disease area in Taiwan. *Archives of Environmental Contamination and Toxicology*, 32, 456–461.
- Huang, Y. K., Lin, K. H., Chen, H. W., Chang, C. C., Liu, C. W., Yang, M. H. et al. (2003). Arsenic species contents at aquacultural farm and in farmed mouthbreeder (Oreochromis Mossambicus) in blackfoot disease hyperendemic areas. *Food and Chemical Toxicology*, 41, 1491–1500.
- Hung, Y. W. (1982). Effects of temperature and chelating agents on cadmium uptake in the American oyster. *Bulletin of Environmental Contamination and Toxicology*, 28, 546–551.
- Kirby, J., Maher, W., Chariton, A., & Krikowa, F. (2002). Arsenic concentrations and speciation in a temperate mangrove ecosystem, NSW, Australia. *Applied Organometallic Chemistry*, 16, 192–201.
- Kohlmeyer, U., Kuballa, J., & Jantzen, E. (2002). Simultaneous separation of 17 inorganic and organic arsenic compounds in marine biota by means of high-performance liquid chromatography/inductively coupled plasma mass spectrometry. *Rapid Communications in Mass Spectrometry*, 16, 965–974.
- Liao, C. M., & Ling, M. P. (2003). Assessment of human health risks for arsenic bioaccumulation in tilapia (Oreochromis mossambicus) and large-scale mullet (Liza macrolepis) from blackfoot disease area in Taiwan. Archives of Environmental Contamination and Toxicology, 45, 264–272.
- Lin, K. H. (2004). Spatiotemporal distribution and bioaccumulation of arsenic species in the aquacultural ecosys-

tem in the coastal areas of southwestern Taiwan (pp. 176–197). PhD Taiwan: dissertation, Institute of Bioenvironmental Systems Engineering, National Taiwan University.

- Lin, Y. S., & Liang, M. H. (1982). Growth and setting of cultured oyster(Crassostrea Gigas Thunberg) in Putai bay. Bulletin of the Institute of Zoology, Academia Sinica, 21, 129–143.
- Norusis, M. J. (1998). SPSS 8.0, Guide to data analysis. Upper Saddle River, N.J.: Prentice-Hall.
- Oremland, R. S., & Stolz, J. F. (2003). The ecology of arsenic. *Science*, 300, 939–944.
- Sanchez-Rodas, D., Geiszinger, A., Gomez-Ariza, J. L., & Francesconi, K. A. (2002). Determination of an arsenosugar in oyster extracts by liquid chromatography-electrospray mass spectrometry and liquid chromatography-ultraviolet photo-oxidation-hydride generation atomic fluorescence spectrometry. *Analyst*, 127, 60–65.
- Shiomi, K., Suglyama, Y., Shimakura, K., & Nagashima, Y. (1996). Retention and biotransformation of arsenic compounds administered intraperitoneally to carp. *Fisheries Science*, 62, 261–266.
- Taiwan EPA (2002). *River water quality in Taiwan*. Available at http://www.niea.gov.tw/protect/2002/2002-06.htm.
- U.S. EPA (1998). Special report on ingested inorganic arsenic: Skin cancer, nutritional essentiality. Washington, D.C.: U. S. Environmental Protection Agency, 1998. Risk Assessment Forum, EPA-25/3-87-13.
- U.S. EPA (2004). Risk-based concentration table. Region 3, Philadelphia: U.S. Environmental Protection Agency.
- Valette-Silver, N. J., Riedel, G. F., Crecelius, E. A., Windom, H., Smith, R. G., & Dolvin, S. S. (1999). Elevated arsenic concentrations in bivalves from the southeast coasts of the USA. *Marine Environmental Research*, 48, 311–333.
- Vilano, M., & Rubio, R. (2001). Determination of arsenic species in oyster tissue by microwave-assisted extraction and liquid chromatography-atomic fluorescence detection. *Applied Organometallic Chemistry*, 15, 658–666.
- Wilson, E. A., Powell, E. N., Wade, T. L., Taylor, R. J., Presley, B. J., & Brooks, J. M. (1992). Spatial and temporal distributions of contaminant body burden and disease in Gulf of Mexico oyster populations: The role of local and large-scale climatic controls. *HelgolaEnder Meeresunter*suchungen, 46, 201–235.