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Element Concentrations in a Unionid Mussel (*Anodonta woodiana*) at Different Life Stages

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The 'background' levels of 18 elements (Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, and Tl) were measured in unionid mussels (*Anodonta woodiana*) at three different life stages (i.e., glochidia–whole body, juveniles and gravid adults–whole soft tissue). Field–collected adult mussels were cultured in experiment ponds for one year before used for tissue collection. Matured glochidia were collected from the adults for the element measurement and the culture of the juvenile mussels. The results of principal component analysis of the element concentrations indicated a significant life stage–dependent variation of element accumulation. The concentrations of Ca, Fe, Zn, As, Cd, Ni, and Mn were generally higher in the adult mussels than those in the juvenile mussels. However, several elements (e.g., Co, Cu, Mo, Ag) were significantly higher in the juveniles than those in the adults. Manganese appeared to be accumulated at high concentration in all three life stages. The background residue concentrations of heavy metals and toxic elements in the artificially produced mussels were very low, while that contaminated As in gravid adults was still obviously high, suggesting that the formers will be potentially more suitable to be used as the bioindicators for the corresponding elements by wild transplantation or laboratory exposure study rather than the gravid adults.

Key words: *Anodonta woodiana*, element, glochidia, gravid adults, juveniles

INTRODUCTION

Unionid mussels are useful in monitoring temporal and spatial trends of a wide range of persistent pollutants in aquatic environments due to many advantages e.g., a wide geographical distribution, sedentary life style, long lifespan, tolerance to a wide range of contaminants, poor metabolic capacity for pollutants, correlation of pollutant content between organism and habitat, adequate tissue for analyses (Tanabe and Subramanian, 2006). A unionid mussel (*Anodonta woodiana*) has been used as a unique bioindicator for 'Freshwater Mussel Watch' research in the Taihu Lake of China (Yang *et al*., 2005) to assess the contamination of organotins (Yang *et al*., 2008), organochlorines (Bian *et al*., 2009) and heavy metals (Liu *et al*., 2010).

A. woodiana is a worldwide–distributed freshwater mussel (Watters, 1997). In China, this species is a traditional edible mussel and its liposome–incorporated aqueous extracts can be potentially applied as a natural antitumor and immunomodulator formulation (Liu *et al*., 2008). Mussels are filter feeders and therefore clean water by reducing algae, particles and toxic materials in the water column (Kurnia *et al*., 2010). The life history of *A. woodiana*, like most unionoids, includes release of sperm by adult males, uptake of sperm by females, fertilization of ova, release of glochidia from females, and attachment of glochidia to a suitable host fish for transformation into juveniles (Cope *et al*., 2008; Dudgeon and Morton, 1983; 1984).

The utilization of mussel–based Mussel Watch monitoring (both passive biomonitoring and active biosurveillance) is generally dependent on field–collected mussels (Andral *et al*., 2004). However, it is difficult to ensure the collection of mussel samples (mainly soft tissue) with similar age/size. This limitation can significantly influence the results of the bioaccumulation of heavy metals and other elements due to different age or size (Metcalfe–Smith *et al*., 1996). Moreover, wild mussels are sometimes rare, or even absent in study locations (Andral *et al*., 2007; Williams *et al*., 1993). Herein a captive breeding population of *A. woodiana* (including glochidia, juveniles, and gravid adults) has been established by artificial propagation techniques in our research laboratory. The cultured mussels can be subsequently transplanted to field with the same source, age, stage of sexual maturity (Andral *et al*., 2004), and low background levels of heavy metals for pollution monitoring.

There have been numerous reports of heavy metals and other elements in adult mussels (e.g., Ferrington *et al*., 1983; Naimo 1995; Ravera *et al*., 2003a; 2003b; 2005; Usero *et al*., 2005) since the Mussel Watch concept was proposed by Goldberg (1975). However, few of studies have been reported on element bioavailability and bioaccumulation in mussels at the different life stages (Cravo *et al*., 2004). These data from different life stages provide important 'background' levels for interpretation the

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monitoring results using mussels as the bioindicators (Scanes and Roach, 1999). In the present study, we assessed the level and dynamics of 18 elements (Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, and Tl) in *A. woodianain* whole body of glochidia and whole soft tissue of juveniles and gravid adults cultured in our research laboratory.

MATERIALS AND METHODS

Sampling

Glochidia, juveniles (J1, 1–month old; J2, 3.5–month old) and gravid adults of *A. woodiana* were collected from an experiment pond of Nanquan Aquatic Base, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences (NFC) from May 2008 to July 2009. The adults were initially collected from Taihu Lake, which is the third largest freshwater lake in China, and cultured in the pond of the NFC for 1 year before used to produce glochidia and the J1 juveniles and J2 juveniles. Once collected, the specimens were depurated for more than 72 h to eliminate gut contents. After a preliminary shell clean–up, the biometric parameters of each bivalve were measured (Table 1). The soft tissues were separated from the shells with a stainless steel scalpel. The matured glochidia (Table 1) were removed from marsupiums of gravid adults. All samples were frozen and maintained at −20°C pending processing. For analysis, the samples were defrosted and washed repeatedly with Milli–Q water (Millipore Corp., USA, with resistivity: 18.2 MΩ•CM). Because of the small body size, the glochidia were divided randomly into four equal analysis samples. Likewise, the soft tissues of the J1 juveniles and J2 juveniles were divided randomly into three equal analysis samples, respectively. As for the gravid adults, total soft tissues from four adults were sampled individually for chemical analysis. The samples of glochidia and soft tissues of juvenile and adult mussels were weighed and dried firstly at 80°C for 24 h. Each dried sample was pulverized individually in an agate mortar and the resulting homogeneous fine powders stored for subsequent analysis.

Element analysis

Element concentrations of glochidia and gravid adults were measured according to a method modified from the method described in Liu *et al*. (2010). Briefly, approximately 0.1 g of dried sample was weighed in a Teflon tube (San'ai Science Co., Japan) to which 1.5 mL of purified nitric acid was added. After pre–digestion at room temperature overnight, the tubes were treated in a microwave oven for 7 min at 230 W, and this procedure was repeated twice. The resultant solution was diluted to a final volume of 30 mL with Milli–Q water and transferred to an acid washed polypropylene tube.

The concentrations of elements of J1 juveniles and J2 juveniles were determined following the procedure described by Ye *et al*. (2011). Simply, each dry sample (0.1±0.005 g) was placed into a Teflon digestion tube, and 10 mL of purified HNO3 was added. All the samples were then digested for 10 min at 120°C, 15 min at 170°C, and a further 15 min at 170°C using a microwave digestion system (ETHOS A T260, Milestone Inc., Italy), and the resultant solutions were diluted to 200 mL with Milli–Q water.

Concentrations of Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, and Tl were analyzed by an Agilent 7500ce Inductive Coupled Plasma – Mass Spectrometry (ICP–MS, Agilent, USA). Matrix–effects and instrumental drift of the ICP–MS were corrected by Li, Sc, Ge, Y, In, and Bi as the internal standard. Recoveries of metal spiked into water samples (n=3) and analyzed by quantitative ICP–MS ranged from 81.3 to 133.7%. All concentration data were expressed as μg/g dry weight.

Data analysis

Statistical analyses were performed using the program SPSS V16.0 (SPSS Inc., USA). A one–way analysis of variance (ANOVA) test was applied to determine the differences between mussel element concentrations at different life stages. A significance level was set up at $P<0.05$. Principal components analysis (PCA) was conducted to evaluate the comprehensive element accumulation pattern of each mussel at different life stages.

RESULTS AND DISCUSSION

In glochidia of *A. woodiana*, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, and Mo were detected while Co, Ag, Cd, and Tl were not detected (Table 2). Essential Ca and Na were the most prominent elements detected, whereas toxic Cr, Ni, As showed the lowest levels. The concentrations of detected elements generally decreased in the order Ca > Na > Mn > K > Mg ≈

Table 1. Biometry data (mean±SD) of glochidia, juveniles and gravid adults of *Anodonta woodiana*

Life stage	Age (Month)	No. of individuals	Shell length (mm)	Shell width (mm)	Shell height (mm)	No. of samples for chemical analysis
Glochidia (G)	/	/	0.257±0.008[a]	/	0.290±0.01[a]	4
Juveniles (J1)	1	87	17.5±2.0	4.3±0.7	8.9±1.1	3
Juveniles (J2)	3.5	12	35.8±2.7	12.1±1.7	20.3±1.7	3
Gravid Adults (GA)	36	4	118.7±9.7	47.9±4.1	75.6±3.4	4

[a] Thirty glochidia were measured randomly.

Table 2. Concentrations of various elements in glochidia, and soft tissues of juveniles, and gravid adults of *Anodonta woodiana* (μg/g dry weight). nd, not detected. The mean data with different superscripts in the same row (except for those of Glochidia) are significantly different ($P<0.05$) following an one–way analysis of variance (ANOVA) test

Element	Glochidia		Juveniles				Gravid Adults	
			1–month–age juveniles (J1)		3.5–month–age juveniles (J2)			
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Na	1819±610	1314–2706	164±48^{a}	114–209	589±64^{b}	537–660	1055±113^{c}	957–1172
Mg	183±77	120–294	825±40	786–866	773±69	710–846	900±122	727–1012
Al	60±15	40–76	328±210	198–570	525±564	106–1167	62±33	36–109
K	232±263	64–623	nd	/	210±54^{a}	148–243	1622±235^{b}	1363–1933
Ca	438625±66226	343700–487100	24264±1563^{a}	23310–26068	16415±1083^{b}	15233–17360	29963±13100^{ab}	15120–43250
Cr	0.5±0.5	nd–1.0	nd	/	nd	/	nd	/
Mn	417±48	369–482	3479±194	3335–3699	2933±241	2783–3211	3560±1218	2076–4659
Fe	180±27	164–221	563±82	509–657	753±794	264–1670	1089±296	840–1486
Co	nd		25±0.7^{a}	24–25	25±0.1^{a}	24.9–25.2	nd	/
Ni	0.9±0.4	0.4–1.2	nd	/	nd	/	0.4±0.1^{b}	0.3–0.5
Cu	17±3.3	15–22	29±0.7^{a}	28–30	28±0.2^{a}	27.8–28.1	13±5.9^{b}	8.4–21
Zn	78±15	64–99	234±11^{a}	225–246	144±12^{a}	132–156	606±208^{b}	327–817
As	5.0±2.3	3.3–8.2	nd	/	nd	/	8.2±0.8^{b}	7.3–9.3
Se	0.8±0.2	0.7–1.1	nd	/	nd	/	2.6±0.4^{b}	2.1–3.0
Mo	0.02±0.03	nd–0.06	28±0.7^{a}	27–29	28±0.3^{a}	28–28.5	0.08±0.06^{b}	0.02–0.2
Ag	nd	/	29±0.8^{a}	28–30	29±0.3^{a}	29–30	nd	/
Cd	nd	/	nd	/	nd	/	0.7±0.4^{b}	0.3–1.4
Tl	nd	/	1.6±0.05^{a}	1.6–1.7	1.6±0.01^{a}	1.6–1.7	nd	

Fe > Al > Zn > Cu > As > Ni ≈ Se > Cr > Mo. Glochidia, consist of shell valves with hook, adductor muscles, mantle, and byssus (Fisher and Dimock, 2002), are the unique larval life stage of most freshwater mussels in the family Unionidae (Haag and Warren, 1999). Brooding through matrotrophy by adult mussels provides nutritional and physiological support for larvae within the marsupium (Watters, 2007), and isolates them from potentially unfavorable water habitats (Schwartz and Dimock, 2001). However, to our knowledge, no data on the maternal–glochidial transfer and bioaccumulation of trace elements are available in mussel at this stage. Our findings that the bioavailability of Ca, Na, Mn, K, Mg, Fe, Al, Zn, Cu, Ni, Se, Cr, Mo, and As to glochidia of *A. woodiana* suggested maternal–glochidial transfer of not only essential major elements (e.g., Ca, Na, K, Mg, Fe) and trace elements (e.g., Mn, Zn, Cu, Se, Mo), but also nonessential (e.g., Al) and toxic elements (e.g., As, Cr, Ni). Therefore, the aforementioned marsupium might not protect glochidia against the transfer and exposure to As, Cr, or Ni when parent mussels are exposed to these toxic elements. Noteworthily, essential Ca and Na were the most prominent elements in glochidia of this study. Calcium is the primary component in mollusc larval shell (Jacob *et al*., 2008; Weiss *et al*., 2002) and involved in the structure of the muscular system and controls essential processes like muscle contraction and cell growth (Belitz *et al*., 2009). Sodium mostly presents as an extracellular constituent and activates some enzymes, such as amylase (Belitz *et al*., 2009).

In the soft tissues of J1 juveniles, the mean concentrations of Ca and Mn were much higher than those of any other elements (Table 2). Chromium, Ni, As, and Cd were not detected and the concentrations of detected elements generally decreased in the order Ca > Mn > Mg > Fe > Al > Zn > Na > Ag ≈ Cu > Mo > Co > Tl > K ≈ Cr ≈ Se (Table 2). In the soft tissues of J2 juveniles, Ca and Mn concentrations were higher than those of any other elements. Likewise in the soft tissues of J1 juveniles, toxic Cr, Ni, As, and Cd were not detected. The concentration order for remaining elements generally decreased as Ca > Mn > Mg ≈ Fe > Na > Al > K > Zn ≈ Ag ≈ Mo ≈ Cu ≈ Co > Tl (Table 2). Therefore, the bioaccumulation pattern of elements could vary during organogenic changes in juveniles of *A. woodiana*, probably relating to some special reasons, e.g., the feeding behavior. By an observation on the morphology and the sequence of organogenesis from juvenile to adult in a similar pearl mussel *Hyriopsis myersiana*, Kovitvadhi *et al*. (2007) found that the ciliary mechanisms around the foot, the mantle, gill, and those on the papillae around the opening of the incurrent siphon were the main feeding behaviors of selective food intake for the 0–40 day–glochidium and 40–>80 day–juvenile, respectively.

In contrast to those in glochidia and juveniles, Ca, Mn, K, Fe and Na were found at higher concentrations than those of any other elements in the soft tissues of gravid adults, while Cr, Co, Ag, and Tl were not detected. It is noteworthy that toxic elements Ni, As, and Cd were detected (Table 2). The concentration order of detected element decreased was Ca > Mn > K > Fe ≈ Na > Mg > Zn > Al > Cu > Se > Cd > Ni > Mo (Table 2). Adult bivalve molluscs have strong ability to filter large volumes of water and obtain food (e.g., detritus, zooplankton, bacteria, algae) principally by filter feeding (Christian and Smith, 2004; Naimo, 1995). As a result, both diet and water can contribute to element accumulation in tissues (Hédouin *et al*., 2007; Pernice *et al*., 2009; Pynnönen, 1991; Wang and Fisher, 1996; 1999a; b), and the diet is considered as a major route (Hédouin *et al*., 2007).

Principal components analysis (PCA) of the element concentrations in the present study was conducted to investigate the comprehensive element accumulation patterns of the mussels at different life stages. The obviously separated scatter plots of scores indicated that there was a significant life history stage–dependent variation of element accumulation among different life stages of the mussel (especially those of glochidia and gravid adults; Fig. 1).

A comparison is made with the mean element concentrations in soft tissues of *Anodonta* mussels at different size in the present study and literature (Table 3). Ca, Fe, Zn, As, Cd, Ni, Mn in *A. woodiana* and other two *Anodonta* mussels were generally higher in the adults than those in the juvenile mussels. However, several elements could obviously higher in the latter than those in the former (e.g., Co, Cu, Mo, Ag). It is noteworthy that Mn was extremely high concentration in every stage individuals of *A. woodiana* in the present study (Table 2), and the freshwater *Anodonta* mussels in previous literatures (Liu *et al*., 2010; Table 3), which was much higher than that of any other elements except for Ca, probably due to that Mn has a special nutritional/physiological function for *Anodonta* mussels, or that the higher concentrations (10–10000 μg/L) of dissolved Mn in freshwater environments (Reimer, 1999). Wenchuan *et al*. (2001) reported that Mn in surface sediments of the Taihu Lake was as high as 360–1500 mg/Kg dry weight.

Table 4 shows the 'background' concentrations of some important heavy metals and toxic elements in tissues of the juveniles and gravid adults of *A. woodiana* mussels from the present study. All element concentrations in the juveniles were far below the residue limits of China, EC and FAO (Table 4) with two exceptions. Zinc concentrations in the adults were within or slightly higher than the range of FAO; As concentration in the adults (1.3 μg/g wet weight) exceeded the limit of China

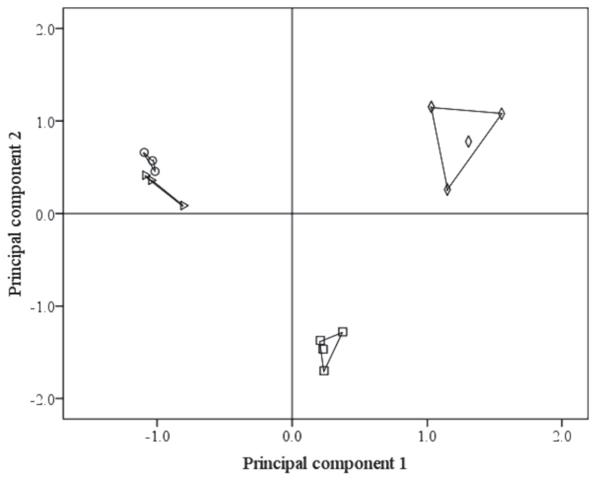

Fig. 1. Scatter plots for scores of principal component analysis in the different stages of *Anodonta woodiana*. Glochidia (square); J1 juveniles (circle); J2 juveniles (triangle); gravid adults (diamond).

Table 3. Comparison of element concentrations (μg/g dry weight) in soft tissues of *Anodonta* mussels from different geographical areas

Location	size[a] (mm)	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Ag	Cd	Tl	References
Anodonta woodiana																
NFC[b], China	17.5	24 264	nd	3 479	563	25	nd	29	234	nd	nd	28	29	nd	1.6	This work
NFC[b], China	35.8	16 415	nd	2 933	753	25	nd	28	144	nd	nd	28	29	nd	1.6	This work
NFC[b], China	118.7	29 963	nd	3 560	1 089	nd	0.4	13	606	8.2	2.6	0.08	nd	0.7	nd	This work
Sanshandao, Taihu Lake, China	102.2		nd	11 237	4 287	nd	nd	19	1 252	15	15	0.3	0.5	22		Liu *et al*. (2010)
Mashan, Taihu Lake, China	97.0		nd	5 921	2 069	nd	nd	18	677	12	14	0.7	0.3	23		Liu *et al*. (2010)
Huzhou, Taihu Lake, China	110.8		nd	11 886	1 977	nd	nd	0.4	661	12	6.7	0.1	nd	0.1		Liu *et al*. (2010)
Dapu, Taihu Lake, China	92.6		nd	8 328	1 386	nd	nd	8.1	912	15	7.8	0.2	0.6	2.5		Liu *et al*. (2010)
Anodonta cygnea																
Ranco Bay, Italy	65–72	74 119	0.4	11 258		1.0	5.0	34	642	13		1.0		10	0.03	Ravera *et al*. (2003)
Anzali wetland, Iran	99–134							0.21						0.1		Pourang *et al*. (2010)
Anzali wetland, Iran	111–140							0.21						0.01		Pourang *et al*. (2010)
Anodonta anodonta																
Dnieper River, Kiev, Ukraine	83–88						0.26[c]/1.9[d]	8.8[c]/36.3[d]	140c/489[d]							Lukashev (2010)
Desna River, Chernigov, Ukraine	92–98						0.43[c]/1.1[d]	3.6[c]/4.7[d]	79c/228[d]							Lukashev (2010)

[a] The mean or range values of shell length, [b] Nanquan Aquatic Base, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, [c] In the clean sections, [d] Downstream near sewage release, nd, not detected.

Table 4. Comparison of concentrations of some heavy metals and arsenic in the soft tissues of juveniles and gravid adults of *Anodonta woodiana* in the present study with related national and international limits (μg/g wet weight)

Element	J1 juveniles[a]	J2 juveniles[a]	Gravid Adults	China	EC[e]	FAO[f]
Cr	nd	nd	nd	2.0[c]		1
Cu	1.0 (0.98–1.05)[b]	1.85 (1.83–1.85)	2.0 (1.3–3.3)	50[d]		10–30
Zn	8.2 (7.9–8.6)	9.5 (8.7–10.3)	94 (51–127)			40–100
As	nd	nd	1.3 (1.1–1.4)	0.5[c]		0.1–5
Cd	nd	nd	0.1 (0.05–0.2)	1.0[d]	1	2

[a] J1 juveniles, 1–month–age; J2 juveniles, 3.5–month–age, [b] Concentration data are presented as mean (range); Wet weight concentration of heavy metals was calculated using the moisture content determined in present study of 96.5%, 93.4%, and 84.5%, respectively, [c] Maximum Levels of Contaminants in Foods, promulgated by Ministry of Health, China, 2005, [d] Residue Limit of Toxic Substances in Nuisanceless Foods and Aquatic Products, promulgated by Ministry of Agriculture, China, 2006, [e] Setting maximum levels for certain contaminants in foodstuffs, promulgated by Commission Regulation, European Communities, 2001, [f] Compilation of legal limits for hazardous substances in fish and fishery products, promulgated by FAO, 1983, nd, not detected.

(0.5 μg/g wet weight), but was within the range of FAO (0.1–5 μg/g wet weight). These results suggest that the background residue concentrations of heavy metals and toxic elements in our artificially produced juvenile mussels were low, and suitable to be used as the bioindicators for the corresponding elements by wild transplantation or laboratory exposure study. The gravid adults were collected from the Taihu Lake and held in the pond at the NFC for 1 year. The high level of As in the adults indicated that these mussels, even living in farmed habitats for a long period, may not be suitable for wild transplantation to monitor the As pollution.

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