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Species, valve size, and pretreatment effects on $\delta^{18}O$ and $\delta^{13}C$ values of ostracod valves from Lake Qinghai, Qinghai–Tibet Plateau

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Abstract

Stable carbon and oxygen isotope ratios of lacustrine ostracod valves are widely used to deduce paleoenvironmental information. We tested the effects of sample pretreatment methods (methanol, deionised water and methanol, and hydrogen peroxide) on ostracod shell oxygen and carbon isotope compositions. In addition, we examined the stable isotope differences among ostracod valves of different sizes and between the two species (*Eucypris inflata* and *Limnocythere inopinata*) present in Lake Qinghai. We found that different treatment methods have no significant effect on the measured C or O stable isotope ratios of ostracod valves, and therefore propose that pretreatment is not needed before isotope analysis. Oxygen isotope ratios of *E. inflata* were positively correlated with *L. inopinata.* This correlation suggests that the $\delta^{18}O$ values of the two species respond similarly to environmental change. The $\delta^{13}C$ values of *E. inflata* showed no correlation with *L. inopinata*, and *E. inflata* had consistently higher $\delta^{13}C$ values than *L. inopinata*. Valve length had no effect on measured $\delta^{18}O$ values for *E. inflata*, but $\delta^{13}C$ values increased with the decreasing shell length (for length classes: <0.8 mm, 0.8–0.65 mm, 0.65–0.5 mm and <0.5 mm). Our results suggest that species and size have very little effect on the oxygen isotope composition of ostracod valves, making it easy to work with ostracod samples when studying lake $\delta^{18}O$ history. In contrast, much more attention must be paid to the selection of species and size when using the carbon isotope composition of ostracods for palaeoenvironmental reconstruction.

Keywords: Ostracod; Species; Size; Carbon; Oxygen; Isotopes; Lake Qinghai

1. Introduction

Stable isotope (C, O) records from fossil ostracod valves deposited in lake sediments are often thought to be ideal for reconstructing paleo-lake water chemistry and temperature, because the oxygen and carbon isotope ratios of valves reflect characteristics of the water in which they grew (e.g. Fritz et al., 1975; Lister, 1988; von Grafenstein et al., 1992, 1994, Schwalb et al., 1994; Keatings et al., 2002). Over the last threedecades, isotope ratios of oxygen and carbon in ostracod valves have been extensively used to reconstruct paleoenvironment (e.g. Fritz et al., 1975; Lister et al., 1991; Xia et al.,

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1997a; von Grafenstein et al., 1999; Schwalb et al., 1999; Heaton et al., 1995; Holmes, 1996; Ingram et al., 1998; Bahr et al., 2006; Bright et al., 2006).

Despite the frequent use of oxygen and carbon isotopes in ostracod-based palaeoenvironmental reconstruction, some observers have found that vital effects have biased the $\delta^{18}O$ and $\delta^{13}C$ values in ostracod valves and that these offsets were different in different species or in different growth-stages of one species (Dettman et al., 1995; von Grafenstein et al., 1999; Holmes and Chivas, 2002; Keatings et al., 2002; Simstich et al., 2004; Scharf et al., 2005). In addition to the question of species-specific offsets, the pretreatment of ostracod samples may have an effect on the measured isotope composition, thereby confounding the interpretation and comparison of these palaeoenvironmental records (Holmes, 1996; Keatings et al., 2006).

Examples of isotopic differences in valves from one location are common. Lister et al. (1991) analyzed co-occurring specimens *Limnocythere inopinata* and *Eucypris inflata* from a core in Lake Qinghai and found that the $\delta^{18}O$ values of *L. inopinata* were 0.4‰ higher than *E. inflata*. They suggested that the $\delta^{18}O$ difference was caused by some degree of segregation between the two species within the ~5-yr sediment sample. Dettman et al. (1995) found that adult and juvenile *Candona subtriangulata* sometimes differed in $\delta^{18}O$ by 2‰, a variation that probably reflects water temperature differences. von Grafenstein et al. (1999) observed that different species of ostracod living in the same lake environment had notably different $\delta^{18}O$ values. They suggested that the $\delta^{18}O$ differences in species were probably caused by "vital offsets", and that these vital offsets seemed to be temperature independent and constant for all instars of a species (von Grafenstein et al., 1999). Keatings et al. (2002) found that *Candona candida* and *Pseudocandona rostrata* had very similar $\delta^{18}O$ and $\delta^{13}C$ values in the same pond, but that *Herpetocypris reptans* had lower $\delta^{18}O$ and $\delta^{13}C$ values. From this they suggested that the fractionation of carbon and oxygen isotope ratios in ostracod valves is related to species, moult stage (juvenile or adult) and water pH. Belis and Ariztegui (2004) observed that there were slight differences in oxygen isotopic composition between adult *Candona neglecta* and *Ilyocypris bradyi*, but that the carbon isotopic composition varied with species and size. Simstich et al. (2004) reported that there was no correlation between ostracod valve size and its isotopic compositions. Scharf et al. (2005) found that the $\delta^{18}O$ values of *C. candida* were more positive than *H. reptans* and *Cypridopsis vidua* in the same interval of a core. They observed that *H. reptans* and *C. vidua* form their carapace primarily in summer, but *C. candida*, which live some millimetres below the sediment surface, tend to form their last instars and adult carapace in the autumn and winter. The $\delta^{18}O$ differences between these three species, therefore, may not be caused by vital offsets, but by differences in seasonal growth patterns or perhaps small-scale habitat differences (Scharf et al., 2005).

It is a common process to pretreat carbonate samples prior to isotopic analysis in order to remove organics and contaminants. Holmes (1996) suggested that the effectiveness of cleaning methods for ostracod valves has not been adequately tested and that further experiments are needed to assess the impact of different cleaning techniques on the geochemistry of ostracod valves. Keatings et al. (2006) recommended minimal pretreatment, although, if needed, hydrogen peroxide treatment and plasma ashing could be used for oxygen isotope analysis, if no other analyses were to be performed. If stable isotope measurement of both C and O isotopes were planned, pretreatment should be limited to plasma ashing only.

The aim of this work was to evaluate the impact of a range of simple pretreatment methods on stable isotope analysis of ostracod valves and to investigate differences in carbon and oxygen isotope ratios between ostracod valves of different size and species. We tested the influence of three different pretreatments (methanol, deionised water and methanol, hydrogen peroxide) on the measured isotopic composition of ostracod carbonate. We also investigated the influence of species and valve size on $\delta^{18}O$ and $\delta^{13}C$ values as a guide to future work on the isotopic composition of these species in core materials.

2. Backgrounds and methods

2.1. Study site

Lake Qinghai, the largest saline lake in China, lies in a closed intermontane basin on the Qinghai Tibet plateau, between 36°32′–37°15′N and 99°36′–100°47′E. In the northeastern portion of the plateau, the lake is influenced by the East Asian summer monsoon, Indian summer monsoon, winter monsoon, and the westerly jet stream. Because of its location at the intersection of numerous important climatic phenomena, it has been the subject of many academic studies. This includes work on sedimentation rate of the lacustrine sediments (Huang, 1988), geochemistry of ostracod shells (Zhang et al., 1989; Lister et al., 1991; Henderson et al., 2003; Liu et al., 2007), carbonate content (Kelts et al., 1989),

isotopic composition of bulk carbonate (Xu et al., 2006), and water chemistry (Zhang, 1994).

2.2. Sampling and methods

Two species of ostracod were used in this study namely *E. inflata* and *L. inopinata*. They are the only two species currently living in Lake Qinghai, based on our observations and other studies (Huang, 1986; Zhang et al., 2006). Samples for this study were taken from the QH-16A core and surface sediments at six different sites in Lake Qinghai. In 1989, using a self-designed platform, a 522 cm long sediment core (QH-16A) was drilled in the southeast part of Lake Qinghai at a water depth of 24 m (Zhang et al., 1989). Ostracod samples were selected from this core at different depths. The surface sediments from other locations were collected in August of 2005 using a self-designed gravity corer (Wang et al., 1998). Few living ostracods were recovered from the sediment surface samples, so empty valves from these samples were used for this study. All ostracod valves were checked for diagenesis with an optical microscope; the empty valves were completely translucent and not affected by diagenesis.

The wet surface sediment and core samples were soaked in deionised water for about 2 h and wet sieved with a 160-mesh sieve. All ostracod valves were dried at room temperature. Empty ostracod valves (*E. inflata* and *L. inopinata*) were then picked using a fine brush and deionised water. Adult *E. inflata* valves were used the study of pretreatment methods. In our study, an *E. inflata* ostracod was considered an adult if the shell length was greater than 0.8 mm.

To evaluate the effect of pretreatment method on the isotopic analysis of ostracod valves, about 120 adult valves were divided into four treatments: 1), not treated, 2), treated with methanol, 3), treated with deionised water and methanol, 4), treated with hydrogen peroxide. To evaluate the effect of size difference on the isotopic composition within one species, *E. inflata* were selected from five stratigraphic levels in the QH-16A core and from the surface sediment samples. Each sample was divided into four groups according to shell length (SL): SL $\geq$0.8 mm, 0.8 mm>SL$\geq$0.65 mm, 0.65 mm>SL$\geq$0.5 mm and SL<0.5 mm. Finally, adult *E. inflata* (SL$\geq$0.8 mm) and *L. inopinata* (SL$\geq$0.6 mm) from the same level in the core and from the surface sediment samples were used to investigate the isotopic differences between species.

Ostracod samples were analyzed for $^{18}O/^{16}O$ and $^{13}C/^{12}C$ at the Institute of Earth Environment, CAS using an isotope ratio mass spectrometer (MAT-252) with an automated Carbonate Preparation Device (Kiel α). The number of ostracod valves used for $^{18}O/^{16}O$ and $^{13}C/^{12}C$ analysis is shown in Table 1. Results were

Table 1
Number of ostracod valves used in each $^{18}O/^{16}O$ and $^{13}C/^{12}C$ analysis

Shell length (mm)	The amount of ostracod valves	Species
SL$\geq$0.8	3–4	*E. inflata*
0.8>SL$\geq$0.65	4–5	*E. inflata*
0.65>SL$\geq$0.5	5–7	*E. inflata*
SL<0.5	6–9	*E. inflata*
SL$\geq$0.6	10–16	*L. inopinata*

Table 2
Results of isotope analysis for ostracods using different pretreatment methods

	Untreated		Methanol		Hydrogen peroxide		Deionised water and Methanol	
	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)
I 1	−1.97	0.88	−2.40	0.95	−1.81	1.01	−2.14	1.03
I 2	−1.74	0.67	−2.35	1.03	−2.21	1.21	−1.71	0.83
I 3	−2.34	0.81	−2.46	1.33	−2.24	1.01	−1.99	1.06
I 4	−2.27	1.36	−2.30	0.85	−2.20	0.77	−1.75	0.74
I 5	−2.14	1.28	−1.95	0.97	−2.14	0.79	−1.68	1.25
Mean	−2.09	1.00	−2.29	1.03	−2.12	0.96	−1.85	0.98
SD	0.22	0.27	0.17	0.16	0.16	0.16	0.18	0.18
II 1	−2.14	0.12	−2.13	0.40	−2.09	0.21	−2.25	0.31
II 2	−2.33	0.29	−2.16	0.40	−2.38	0.35	−2.05	0.46
II 3	−2.39	0.48	−2.16	0.36	−1.81	0.24	−2.09	0.27
II 4	−2.17	0.59	−2.19	0.28	−1.91	0.30	−2.09	0.49
II 5	−2.24	0.62	−2.31	0.21	−2.16	0.30	−2.34	0.39
Mean	−2.25	0.42	−2.19	0.33	−2.07	0.28	−2.16	0.38
SD	0.09	0.19	0.06	0.07	0.20	0.05	0.11	0.08
III 1	−1.96	0.67	−2.01	1.00	−1.84	1.21	−1.59	0.76
III 2	−1.59	0.68	−1.88	0.62	−1.55	1.01	−1.65	0.76
III 3	−1.63	0.59	−1.75	1.14	−1.60	0.73	−1.45	0.61
III 4	−1.61	0.81	−1.83	0.81	−1.56	0.73	−1.69	0.75
III 5	−1.78	1.06	−1.92	1.10	−1.72	0.70	−1.59	0.38
Mean	−1.71	0.76	−1.88	0.93	−1.65	0.87	−1.59	0.65
SD	0.14	0.16	0.09	0.19	0.11	0.20	0.08	0.15
IV 1	3.00	−0.09	3.01	0.13	3.25	0.01	3.10	0.12
IV 2	3.14	0.40	2.95	−0.04	3.06	0.07	3.06	0.53
IV 3	3.28	−0.08	2.87	0.43	3.21	−0.28	3.08	0.15
IV 4	3.07	0.08	2.43	0.12	3.27	−0.31	3.16	0.24
IV 5	3.15	0.44	3.05	0.53	3.11	−0.03	3.15	0.21
Mean	3.14	0.15	2.86	0.23	3.18	−0.11	3.11	0.25
SD	0.09	0.23	0.22	0.21	0.08	0.16	0.04	0.15
V 1	2.53	−1.06	2.08	−0.92	2.19	−1.08	2.01	−1.14
V 2	2.09	−0.89	2.24	−0.65	1.81	−0.84	2.15	−1.22
V 3	2.13	−0.73	1.98	−1.21	1.96	−0.88	2.02	−1.11
V 4	2.38	−0.83	2.02	−1.08	1.85	−0.85	2.04	−0.92
V 5	2.31	−0.75	1.98	−1.01	2.22	−0.98	2.22	−0.96
Mean	2.29	−0.85	2.06	−0.97	2.01	−0.93	2.09	−1.07
SD	0.16	0.12	0.09	0.19	0.17	0.09	0.08	0.11

Table 3
Results of Paired-Sample *T* test for $^{18}O/^{16}O$ analysis using different preparation techniques (N=no treatment, M=methanol treatment, WM=deionised water and methanol treatment, WH=H_2O_2 treatment)

	t	*df*	*P*
N-M	2.786	4	0.05
N-WH	−0.59	4	0.587
N-WM	0.079	4	0.941

expressed in delta (δ) notation relative to the V-PDB standard. Four lab standard carbonates, for which isotope ratios are well known, were also measured in each set of samples. The analytical error of the lab standard is approximately ±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{18}O$.

3. Results

3.1. Pretreatment effects on isotope compositions

We tested the effect of three simple pretreatments on the carbon and oxygen isotope compositions of ostracod valves. Using adult *E. inflata* valves (shell length> 0.80 mm) required ~4 valves for a single $^{18}O/^{16}O$ and $^{13}C/^{12}C$ measurement (Table 1). The results of these $^{18}O/^{16}O$ and $^{13}C/^{12}C$ analyses are given in Table 2. A Paired-Sample *T* test was used to compare the effects of pretreatment on both $\delta^{18}O$ and $\delta^{13}C$ (Tables 3 and 4).

One pretreatment, methanol, showed a significant difference in $\delta^{18}O$ ($t=2.786$, $p=0.05$). For the other two, H_2O_2 and deionised water/methanol, there was no significant difference ($t=-0.590$, $p=0.587$ and $t=-0.079$, $p=0.941$, respectively). No significant difference in carbon isotope ratio was shown by any pretreatment (methanol, $t=-0.261$, $p=0.807$; H_2O_2, $t=1.099$, $p=0.333$; methanol/deionised water, $t=1.351$, $p=0.248$).

The very small error bars about each mean $\delta^{18}O$ in Fig. 1a showed that the $\delta^{18}O$ results of each sample were very reproducible. Although we find a significance level of $p=0.05$ after the Paired-Sample *T* test for the $\delta^{18}O$ of untreated samples vs. methanol treated samples, the maximal shift of mean $\delta^{18}O$ between untreated samples and methanol treated samples is not more than 0.3‰ (Table 3). So, we conclude that only very minor evidence exists for a $\delta^{18}O$ difference between untreated samples and methanol treated samples.

From the results of the statistical tests and Fig. 1b, we conclude that there are no $\delta^{13}C$ differences between untreated samples and treated samples for any pretreatment. Although the error of the mean $\delta^{13}C$ values in Fig. 1b was slightly larger than the analysis error of the lab standard (±0.1‰ for $\delta^{13}C$), the results can be used to evaluate influence of pretreatment on the $\delta^{13}C$ value of ostracods.

3.2. Size effects on isotopic composition

Because fully adult ostracod valves are not always available in cores, we tested the effects of valve size on isotopic ratio. We divided ostracod valves into four groups according to their shell length. Valves with shell length exceeding 0.80 mm were considered adults and were labeled A. Valves with lengths from 0.80 mm to 0.65 mm were labeled B. Similarly, the C valves were from 0.65 mm to 0.50 mm, and the D valves were less than 0.5 mm. We randomly selected ostracod valves from each size range for carbon and oxygen isotope analysis, the results are given in Table 5.

Our results indicate that there are no significant differences in mean $\delta^{18}O$ values of *E. inflata* for different shell lengths within the standard error (Fig. 2a). But a significant change in $\delta^{13}C$ occurred for each sample set as valve length changed. The $\delta^{13}C$ trend from largest to smallest valve in each group was from +0.33‰ to +1.2‰ (QH1), +2.38‰ to +3.08‰ (S-16), +0.98‰ to +1.93‰ (S-22), +0.33‰ to +1.28‰ (S-24),−0.58‰ to +0.34‰ (S−48) (Fig. 2b). The $\delta^{13}C$ shifts range from 0.95‰ to 0.7‰ between largest valves and smallest valves. For all five groups of ostracod samples, the $\delta^{13}C$ value became more positive with a decrease in shell length (Fig. 2b).

3.3. The isotopic compositions of different species

Adult *E. inflata* (SL≥0.8 mm) and *L. inopinata* (SL≥0.6 mm) from the same level in the core and from the surface sediment samples were used to investigate the isotopic differences between species. Results of isotope analysis for *E. inflata* and *L. inopinata* from QH-16A and the surface sediments are given in Tables 6 and 7.The range of $\delta^{18}O$ values in core QH-16A are −0.36‰ to +3.4‰ for *E. inflata* and + 0.31‰ to +3.37‰ for *L. inopinata* (Fig. 3a). In the six surface

Table 4
Results of Paired-Sample *T* test for $^{13}C/^{12}C$ analysis using different preparation techniques (N=no treatment, M=methanol treatment, WM=deionised water and methanol treatment, WH=H_2O_2 treatment)

	t	*df*	*P*
N-M	−0.261	4	0.807
N-WH	1.099	4	0.333
N-WM	1.351	4	0.248

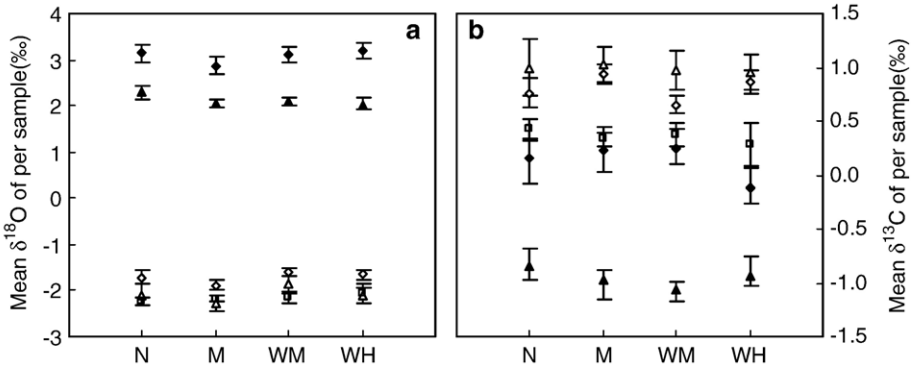

Fig. 1. Pretreatment of ostracod samples from core and surface sediments: I (△), II (□), III (◇), IV (◆), V (▲). Treatments were: *N*=no treatment, M=methanol treatment, WM=deionised water and methanol treatment, WH=H_2O_2 treatment. a) Mean $\delta^{18}O$ values for each treatment within each sample; b) Mean $\delta^{13}C$ values for each treatment within each sample. Vertical bars are standard deviation (n=3 to 5).

sediment samples $\delta^{18}O$ ranges from +2.02‰ to +4.53‰ for *E. inflata* and from 2.07‰ to 3.77‰ for *L. inopinata* (Fig. 2b). Oxygen isotope ratios of the two species were positively correlated with each other in both the core samples (p <0.01, R^2=0.73) and the sediment surface samples (p <0.05, R^2=0.71) (Fig. 3). This correlation suggests that the $\delta^{18}O$ values of the two species respond to environmental changes in Lake Qinghai. Carbon isotope ratios range from −0.63‰ to +2.38‰ for *E. inflata*, and from −2.35‰ to −0.45‰ for *L. inopinata* in the QH-16A core (Fig. 4a). For the surface sediment samples, $\delta^{13}C$ ranges from −0.31‰ to +0.3‰ for *E. inflata* and from −1.06‰ to −0.54‰ for *L. inopinata* (Fig. 4b). There is no correlation between the $\delta^{13}C$ of *E. inflata* and the $\delta^{13}C$ of *L. inopinata* in either the core of surface sediment samples (Fig. 4). *E. inflata* had consistently higher $\delta^{13}C$ values than *L. inopinata* (Fig. 5), with an offset between the two species of +0.45‰ to +2.35‰ in different sections from the core (Fig. 5a). In the six surface sediment samples, *E. inflata* had $\delta^{13}C$ values +0.54‰ to +1.06‰ higher than those of *L. inopinata* (Fig. 5b).

4. Discussion

4.1. The effect of pretreatment on isotopic composition

There were no significant differences in carbon and oxygen isotopic compositions between treated and untreated ostracod valves. This result is consistent with

Table 5
Results of isotope analysis for ostracods with different shell length

		SL≥0.8 mm		0.8 mm>SL≥0.65 mm		0.65 mm>SL≥0.5 mm		SL<0.5 mm	
		$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)
S-16	Mean	0.10	2.38	0.18	2.38	−0.2	2.91	−0.27	3.08
	SD	0.22	0.08	0.11	0.37	0.16	0.23	0.16	0.09
	n	4	4	4	4	4	4	4	4
S-22	Mean	−1.85	0.98	−2.14	0.85	−2.31	1.63	−2.18	1.93
	SD	0.18	0.17	0.08	0.07	0.09	0.13	0.09	0.07
	n	5	5	4	4	4	4	3	3
S-24	Mean	−2.17	0.32	−2.27	0.59	−2.4	0.88	−2.36	1.28
	SD	0.17	0.27	0.32	0.26	0.12	0.1	0.10	0.11
	n	7	7	4	4	5	5	4	4
S-48	Mean	−2.82	−0.58	−2.58	0.21	−2.53	0.13	−2.75	0.34
	SD	0.52	0.23	0.27	0.23	0.15	0.09	0.10	0.09
	n	6	6	8	8	8	8	4	4
QH-1	Mean	3.12	0.33	3.12	0.63	3.08	1.01	3.11	1.20
	SD	0.10	0.08	0.26	0.18			0.09	0.04
	n	2	2	2	2	1	1	2	2

Note. *n* is number of repeat analysis for each samples.

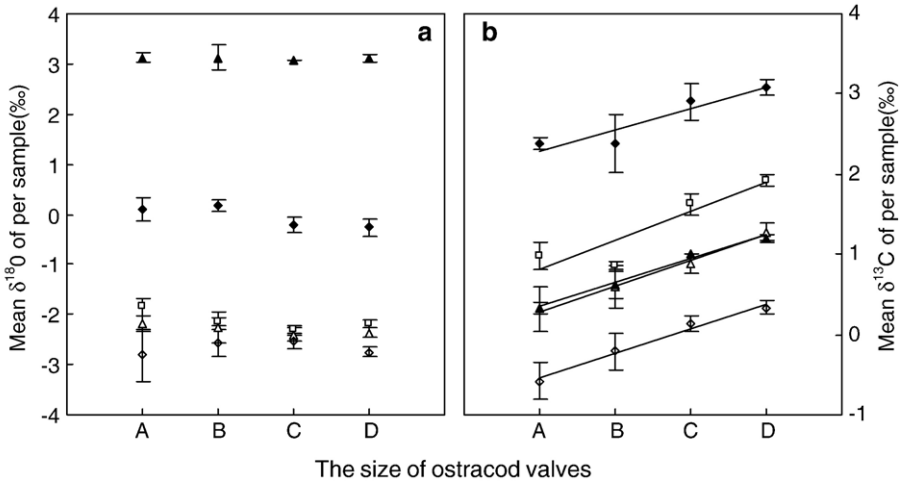

Fig. 2. The mean $\delta^{18}O$ and $\delta^{13}C$ values of valves with different shell length (A, B, C, D represent SL ≥ 0.8 mm, 0.8 mm > SL ≥ 0.65 mm, 0.65 mm > SL ≥ 0.5 mm and SL < 0.5 mm respectively). a) Mean $\delta^{18}O$ values of valves with different shell length; b) Mean $\delta^{13}C$ values of valves with different shell length. Five groups of ostracod samples are from the core and surface sediment: QH01 (▲), S-16 (♦), S-22 (□), S-24 (△), S-48 (◊). Vertical bars are standard deviation (n = 3 to 5).

pretreatment experiments for elemental and isotopic analysis of ostracod valves by Keatings et al., 2006. There are a number of possible reasons for the lack of measurable change in isotopic composition: 1) The valves used in this study had been adequately cleaned during the initial treatment with deionised water when adhering sediments were removed; 2) Organic matter may not react with 100% phosphoric acid at 70°C and thus not release contaminating CO_2 for the isotopic analysis; 3) The amount of organic material remaining in the ostracod valves is perhaps too small to affect the $\delta^{13}C$ value. Because the $\delta^{18}O$ and $\delta^{13}C$ values of organic matter are typically lower than calcite, the $\delta^{13}C$ values of ostracod valves should increase if organic matter was removed by methanol or hydrogen peroxide (Keatings et al., 2006). However, in our results the $\delta^{13}C$ values of ostracod samples decreased slightly after organic matter was removed. Our result is similar to that of Grottoli et al. (2005), which found that, in most cases, the $\delta^{13}C$ values of coral samples decreased significantly when organic matter was removed. They suggested that organic carbon in the coral skeleton or in polyp tissues is not a source of contamination in the measurement of skeletal $\delta^{13}C$ values.

Because these pretreatments have no observable effect on the isotopic composition of ostracod valves, we suggest that pretreatment is not needed for ostracods in this state of preservation. There are significant advantages to working in a system such as this: (1) samples loss and recontamination is avoided in the pretreatment step; (2) the workload is reduced and sample preparations are greatly simplified.

4.2. The isotopic differences by size class

The $\delta^{18}O$ of biogenic carbonate formed in lake water is mainly controlled by the $\delta^{18}O$ value of the ambient

Table 6
Results of isotope analysis for *E. inflata* and *L. inopinata* from QH16-A core

		E. inflata		*L. inopinata*	
		$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)
S-1	Mean	3.18	0.74	3.37	−0.45
	SD	0.14	0.10	0.07	0.10
	n	2	2	2	2
S-4	Mean	3.27	−0.46	3.35	−1.16
	SD	0.25	0.61	0.13	0.12
	n	2	2	2	2
S-12	Mean	3.40	0.06	2.98	−1.74
	SD	0.09	0.02	0.25	0.23
	n	3	3	3	3
S-14	Mean	1.75	0.46	0.51	−1.71
	SD	0.20	0.28	0.09	0.13
	n	3	3	3	3
S-16	Mean	0.10	2.38	0.35	−2.10
	SD	0.22	0.08	0.16	0.14
	n	4	4	3	3
S-18	Mean	−0.36	−0.63	0.31	−2.35
	SD	0.20	0.39	0.17	0.09
	n	3	3	2	2
S-59	Mean	0.01	0.05	0.68	−1.37
	SD	0.18	0.10	0.05	0.09
	n	4	4	3	3
S-65	Mean	0.42	2.06	2.08	−1.77
	SD	0.64	0.11	0.10	0.49
	n	2	2	2	2

Note. *n* is number of repeat analysis for each samples.

Table 7
Results of isotope analysis for *E. inflata* and *L. inopinata* from the surface sediments

		E. inflata		*L. inopinata*	
		$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)
QH-1	Mean	3.11	0.25	3.26	−0.56
	SD	0.04	0.15	0.15	0.14
	n	5	5	5	5
QH-2	Mean	2.69	0.30	2.43	−0.72
	SD	0.22	0.21	0.13	0.06
	n	5	5	5	5
QH-3	Mean	2.02	−0.02	2.07	−1.06
	SD	0.11	0.06	0.08	0.10
	n	4	4	6	6
QH-4	Mean	4.53	−0.31	3.29	−0.86
	SD	0.10	0.14	0.06	0.13
	n	3	3	2	2
QH-5	Mean	3.41	−0.03	3.53	−0.54
	SD	0.44	0.14	0.19	0.09
	n	3	3	2	2
QH-6	Mean	4.35	−0.24	3.77	−0.59
	SD	0.38	0.27	0.07	0.05
	n	3	3	2	2

Note. *n* is number of repeat analysis for each samples.

water and the temperature at which the carbonate formed, in addition, biological factors (vital offsets) may play a role (Craig, 1965; Stuiver, 1970). Xia et al. (1997b) found that faster calcification lead to more incorporation of ^{18}O relative to inorganic calcite and temperature was negatively correlated with the $\delta^{18}O$ values of ostracod valves. von Grafenstein et al. (1999) found that the $\delta^{18}O$ of the A-1 instar of *Candona* sp. was depleted by 1‰ relative to adults at one station and was nearly identical to the $\delta^{18}O$ of adults at deeper water stations. The $\delta^{18}O$ values of both *C.* sp. A-1 instars and adults were different from the expected $\delta^{18}O_{eq}$ of inorganic calcite. They argued that this difference could not be explained by seasonal temperature effects or variation in the $\delta^{18}O$ of ambient water, rather the differences were caused by additional effects related to biologically mediated calcification of the valves. A-1 instars of Candoninae begin to appear after the beginning of cooling in the autumn, and adults dominate from late autumn to early spring (von Grafenstein et al., 1999). If there is a constant non-temperature dependent 'vital offset' (von Grafenstein et al., 1999), the similar $\delta^{18}O$ values of *C.* sp. A-1 instars and adults may be due to different calcification times or the effects of different calcification rates. However, our data shows that there is no significant difference in mean $\delta^{18}O$ value between juvenile and adult valves in modern Lake Qinghai (Fig. 2).

If the vital offsets are constant for all instars of a species (von Grafenstein et al., 1999), then they play no role in the $\delta^{18}O$ history of a lake derived from ostracod shell carbonate provided only a single species is used. The oxygen isotopic compositions of all instars are mainly influenced by water temperature and water $\delta^{18}O$. In the spring, when the temperature of lake water starts to rise, *E. inflata* forms juvenile valves. Adult ostracod valves are formed in summer, at high temperatures in Lake Qinghai. If we assume that the $\delta^{18}O$ value of water remains constant throughout the year, the $\delta^{18}O$ values of juveniles should be more positive than the adult valves, because of their higher rates of calcification and the lower temperatures at which they calcify, both of which favor more positive $\delta^{18}O$ values. But in the arid to semiarid Lake Qinghai region, where evaporation is usually greater than precipitation, the $\delta^{18}O$ value of lake water may shift to more positive values during the hot summer months. In other words, high summer temperatures can result in both a positive (evaporation) and negative (temperature effect on fractionation factor) shift in the $\delta^{18}O$ values of ostracod valves. These two factors work in opposite directions, but if the evaporative increase in water $\delta^{18}O$ exceeds the magnitude of the

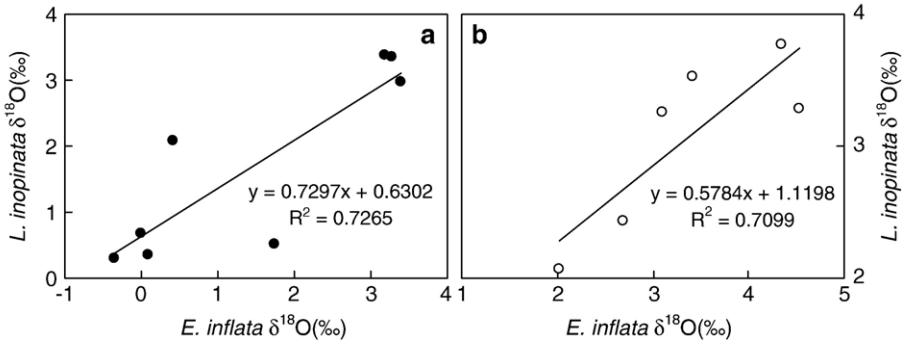

Fig. 3. Correlation of $\delta^{18}O$ values between *L. inopinata* and *E. inflata*. a) Linear correlation of $\delta^{18}O$ values between *L. inopinata* and *E. inflata* from QH16-A core. b) Linear correlation of $\delta^{18}O$ values between *L. inopinata* and *E. inflata* from the surface sediments.

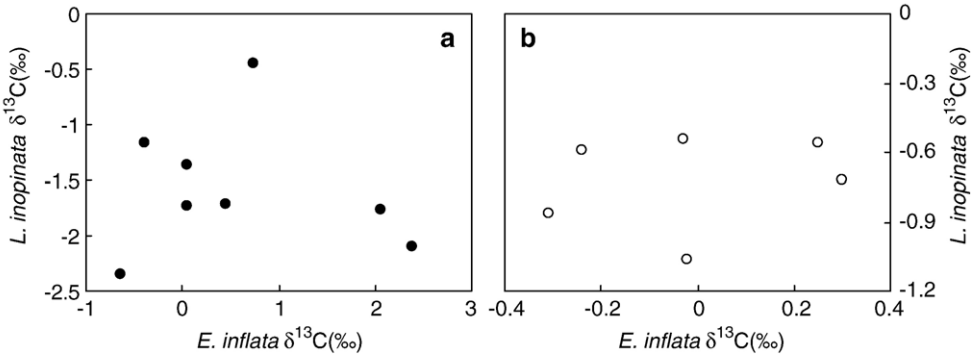

Fig. 4. Relationship of $\delta^{13}C$ values between *L. inopinata* and *E. inflata*. a) Relationship of $\delta^{13}C$ values between *L. inopinata* and *E. inflata* from QH16-A core. b) Relationship of $\delta^{13}C$ values between *L. inopinata* and *E. inflata* from the surface sediments.

negative shift in ostracod $\delta^{18}O$ caused by increasing temperature, the $\delta^{18}O$ of ostracod valves will become more positive. In our results, juvenile and adult valves have very similar $\delta^{18}O$ values. It is possible that the temperature effect on the $\delta^{18}O$ of *E. inflata* may be equal to the $\delta^{18}O$ shift caused by the "vital effects" such as the rate of calcification. Our results, showing no difference between juveniles and adults, simplify the process of ostracod selection when using the $\delta^{18}O$ of *E. inflata* for palaeoenvironmental reconstruction.

Ostracod valve carbon isotope ratios ($\delta^{13}C$) vary in a more complex fashion than $\delta^{18}O$. The carbon isotope value of DIC is much more variable than oxygen isotope composition of the lake water on a micro-scale even in a well-mixed lake. So, $\delta^{13}C$ in ostracod shells should be more variable than oxygen isotope ratios on long time scales. From Fig. 2, we can see that the $\delta^{13}C$ variation of different size classes of ostracod is much larger than $\delta^{18}O$ variation, but that the range in $\delta^{13}C$ values for each size class is much smaller than the range in $\delta^{18}O$ values for the classes. Although we expected greater overall variability in carbon isotope values in ostracod shells than in oxygen isotope ratios, this was not the case. Perhaps microhabitat variations are not important in Lake Qinghai.

Fig. 2 indicates that the $\delta^{13}C$ values of juveniles are more positive than adults (0.7 to 0.95‰). Some studies have indicated that the carbon in ostracod valve carbonate is directly taken up from dissolved inorganic carbon (DIC) (Holmes, 1996; Wansard et al., 1998; von Grafenstein et al., 1999; Schwalb et al., 1999). Variation in the $\delta^{13}C$ of dissolved inorganic carbon in a lake is mainly controlled by lake productivity (Li and Ku, 1997).

Nutrient concentrations in sediment pore waters are 13–15 times higher than nutrients in the Lake Qinghai

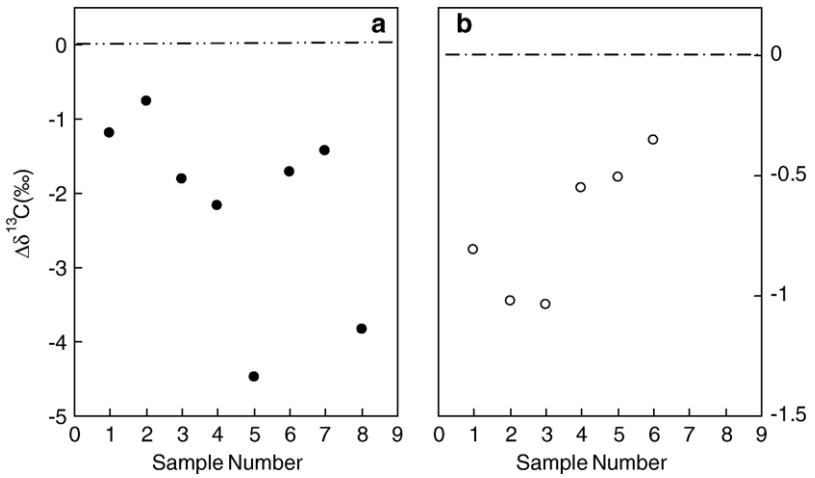

Fig. 5. Differences in $\delta^{13}C$ values between *L. inopinata* and *E. inflata* ($\Delta\delta^{13}C=\delta_{L.\cdot inopinata}{}^{13}C-\delta_{E.\cdot inflata}{}^{13}C$). a) Differences in $\delta^{13}C$ values between *Limnocythere inopinata* and *E. inflata* from the QH16-A core. b) Differences in $\delta^{13}C$ values between *L. inopinata* and *E. inflata* from the surface sediments.

water column (Yang and Wang, 1997). Although phytoplankton concentrations are low in Lake Qinghai, there is abundant benthic algae on the lake bottom, and bottom sediments are rich in organic matter (Yang and Wang, 1997). During algal blooms, alga preferentially take up light carbon during photosynthesis, causing the residual inorganic carbon to become enriched in ^{13}C. Algal respiration produces CO_2 which is depleted in ^{13}C (Stiller and Hutchinsons, 1980), and the decomposition of organic matter yields DIC which is depleted in ^{13}C. When the combined isotopic effects of respiration and organic matter decomposition exceed the effect of photosynthesis, the $\delta^{13}C$ of DIC will become more negative. If juvenile *E. inflata* calcify their valves during a time of high productivity and algal blooms, and adult valves are calcified when respiration exceeds photosynthesis, juvenile *E. inflata* will have higher $\delta^{13}C$ than adults.

Because some studies have suggested that oxygen isotope fractionation in ostracod valves may increase with higher calcification rate (Xia et al., 1997b), it is possible that a disequilibrium effect is also present for carbon isotope ratios. This is also a possible explanation for our observed pattern, if $\delta^{13}C$ is shifted to higher values with increasing rate of calcification, juvenile ostracod valves formed at higher rates of calcification than adults will have higher $\delta^{13}C$ values.

In conclusion, the pronounced difference in $\delta^{13}C$ values of adult and juvenile ostracod valves may reflect seasonal changes of DIC and the productivity of the lakes.

4.3. Isotopic differences between *E. inflata* and *L. inopinata*

Our measured $\delta^{18}O$ values of *E. inflata* are significantly different than those of *L. inopinata*. Although the oxygen isotope ratio of ostracod valves depends primarily on the isotopic composition and temperature of the water in which the valves grew (Xia et al., 1997b), this information is not well known for the Lake Qinghai species. There is little information on the ecology of these ostracods or on seasonal changes in the $\delta^{18}O_{water}$ of Lake Qinghai. We do not know the timing of calcification for these two species or the $\delta^{18}O_{water}$ in which the ostracod valves formed. von Grafenstein et al. (1999) found that ostracods of different species had different 'vital offsets' in $\delta^{18}O$. The difference in vital offset between species caused ostracods living in the same water bodies to have different $\delta^{18}O$ values. Because temperature and $\delta^{18}O_{water}$ may vary with the season, ostracod valves calcified in different seasons should have different $\delta^{18}O$ values. In accordance with previous studies, the $\delta^{18}O$ differences between *E. inflata* and *L. inopinata* in Lake Qinghai may be caused by the "vital offsets" in species, seasonal difference in calcification temperature, or other factors.

In our results, the $\delta^{18}O$ values of *E. inflata* were positively correlated with *L. inopinata*. This correlation suggests that the oxygen isotopic compositions of two species respond congruently to environmental changes. Within each individual sample the $\delta^{18}O$ difference between *L. inopinata* and *E. inflata* ranges from −1.24‰ to +1.66‰ (average is +0.26‰) in the core, and from −1.24‰ to +0.15‰ (average is −0.29‰) in the surface sediments. Lister et al. (1991) found that the $\delta^{18}O$ values of *L. inopinata* were 0.4‰ higher than *E. inflata* from a short-core sample in Lake Qinghai. They suggested that this difference could be due to some temporal segregation in calcification between the two species within the sediment sample which spanned ~ fiveyears of sedimentation. Although our results are quite variable, the mean $\delta^{18}O$ of *L. inopinata* is 0.26‰ higher than *E. inflata* in the core samples, roughly in agreement with Lister et al., 1991. But, in contrast, the mean $\delta^{18}O$ of *L. inopinata* is 0.29‰ lower than *E. inflata* in our surface sediment samples. Because of the high variance between and within our two sample sets (Fig. 3), we are unable to quantify the $\delta^{18}O$ difference between these two species. Additional study is clearly needed, ideally using living *E. inflata* and *L. inopinata* from different sites in Lake Qinghai.

The differences in $\delta^{18}O$ between two species may reflect seasonal environment changes (water $\delta^{18}O$ and temperature) in Lake Qinghai. At present, although we cannot quantify the oxygen isotope "vital offset" between the two species, the positive correlation in $\delta^{18}O$ between *E. inflata* and *L. inopinata* shows that they respond to environmental change. Therefore the $\delta^{18}O$ of two species can be discussed without adjustment for a systematic $\delta^{18}O$ difference when the $\delta^{18}O$ of two species are used for palaeoenvironmental reconstruction in Lake Qinghai.

No clear correlation exists between the $\delta^{13}C$ of *E. inflata* and *L. inopinata*. However, the $\delta^{13}C$ of *E. inflata* was consistently higher than that of *L. inopinata* (Fig. 5). The $\delta^{13}C$ of ostracod is thought to be controlled by the $\delta^{13}C$ of the dissolved inorganic carbonate (DIC) in ambient lake water (von Grafenstein et al., 1999), although Keatings et al. (2002) suggested that the $\delta^{13}C$ of ostracod valves might be related to the pH of surrounding water. Therefore, when discussing the $\delta^{13}C$ record of *E. inflata* and *L. inopinata* valves, there are multiple possible causes for variation: carbon fractionations of the two species ascribed to 'vital offsets', differences in microenvironment, changes of

$\delta^{13}C_{DIC}$, and the pH of the lake water. When using the $\delta^{13}C$ of ostracods from lake cores to study palaeoproductivity, we must pay careful attention to carbon isotope "vital offsets" in different species.

5. Conclusion

Our study of three different pretreatment methods (methanol, DI water and methanol, H_2O_2) was unable to demonstrate a significant effect on C, O stable isotopic analysis of ostracod valves from surface sediments or cores. Therefore we suggest that, for lacustrine ostracods preserved like those of this study (empty completely translucent valves), pretreatment is not needed for stable isotope analysis. This leads to significant advantages: (1) sample loss and recontamination is avoided; (2) workload is significantly reduced and sample preparation is simplified.

Our data showed that the oxygen isotope composition of *E. inflata* valves did not change significantly with varying body length. In contrast, carbon isotope ratios in *E. inflata* increased with a decrease in body length. At present, if carbon and oxygen isotope compositions of *E. inflata* are used to reconstruct environmental history, ostracod valves do not have to be limited to one length class, but the carbon isotope differences between different sizes must be taken into account.

The $\delta^{18}O$ values of *E. inflata* were positively correlated with *L. inopinata*. This correlation suggests that the oxygen isotopic compositions of the two species have similar responses to environmental change. Differences in $\delta^{18}O$ between these two species may reflect seasonal environmental change in Lake Qinghai. The $\delta^{13}C$ of *E. inflata* were consistently higher than *L. inopinata.* The variable $\delta^{13}C$ difference between the two species may reflect changes of productivity in different seasons in Lake Qinghai. If the $\delta^{13}C$ of ostracod valves are used to discuss changes in lacustrine palaeoproductivity, one must consider the carbon "vital offsets" of different species.

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