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Full Length Research Paper

Mineralogical and geochemical study of carp otoliths from Baiyangdian Lake and Miyun Water Reservoir in China

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Carp otoliths from two different freshwaters (Baiyangdian Lake and Miyun Water Reservoir) were mineralogically and chemically analyzed. The water quality standard of Miyun Water Reservoir is Grade 2 which is much better than the Grade 5 of Baiyangdian Lake. The aim of this study was to examine the differences in otoliths in mineralogy and chemistry from the two sites with quite different qualities. All the analyzed carps showed lapillus and sagitta otoliths made of aragonite, except for B-22 (from Baiyangdian Lake) whose lapillus consisted of vaterite and sagitta consisted of aragonite and vaterite; all asteriscus are composed of vaterite. It is inferred that the occurrence of vaterictic otoliths is linked to poor water quality. Chemical analysis showed that significant difference of Pb concentration between sites was tested by *t*-test of the compare means (*t*-test comparison: t = 2.043, $P<0.05$). While the site-specific differences of the other metals were not significant. In addition, a significant difference of Sn concentration was tested as well (*t*-test comparison: t = 2.652, $P<0.05$). Average content of lapilli Pb is consistent with the water dissolved Pb measurement, with higher dissolved Pb concentration in Baiyangdian Lake relative to the Miyun Water Reservoir.

Key words: Carp otoliths, water quality, mineralogy, chemistry, Pb.

INTRODUCTION

Otoliths are the calcified structures in the inner ear of teleost fish that function in balancing and hearing. Most teleost fish have three pairs of otoliths: lapillus, sagitta and asteriscus (Panella, 1980). Otolith calcium carbonate is precipitated from the endolymph in increments throughout the life of a fish (Romanek and Gauldie, 1996). Otoliths have been widely studied so far, there are four principal aspects, which are: (1). Estimating age and daily-annual increments of teleost fishes (Secor et al., 1995; Allain and Lorance, 2000; Cailliet et al., 2001); (2). Mineral composition of lapillus, sagitta and asteriscus in different species (Degens et al., 1969; Gauldie, 1993; Li et al., 2007; Motta et al., 2009); (3). Minor and trace element composition of $CaCO_3$ and influence of ambient water environment (Edmonds et al., 1989; Campana, 1999; Bath et al., 2000; Ranaldi and Gagnon, 2008, 2010); (4). Isotope fractionation of aragonite otoliths and the correlation with temperature and salinity variations of sea water (Devereux, 1967; Gao and Beamish, 2003; Newman et al., 2010; Collingsworth et al., 2010). In terms of mineral polymorph, the calcium carbonate has three crystalline phases: calcite, aragonite and vaterite, in which vaterite is seldom found in the natural environment because it is less stable than the other two calcium carbonate polymorphs. Aragonite is the most common crystal morph of calcium carbonate found in otoliths, calcite and vaterite have been reported in teleost otoliths as well (Degens et al., 1969; Morales-Nin, 1985; Strong et al., 1986; Gauldie and Nelson, 1990; Gauldie, 1986, Tomas, 2004; Sweeting et al., 2004; Yang et al., 2007;

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Jessop et al., 2008). The abnormal otoliths were addressed because of the replacement polymorph in sagitta (Ma et al., 2008), and most lapillus and sagitta are composed of aragonite, whereas asteriscus consists of vaterite (Motta et al., 2009; Li and Feng, 2007). The occurrence of vateritic carbonate was reported not only in fish otoliths, but also in the shell of freshwater snail and cultured pearls recently (Spann et al., 2010; Ma and Lee, 2006).

The otoliths shows the environmental exposure history of fish due to the advantages of biological inertia and rhythm and have the potential to provide the valuable source information of ambient water environment (Li et al., 2007, 2008; Gao et al., 2008). Trace element analyses of otoliths may provide trace element compositions of inhabited environment where other environmental proxies are not available (Edmonds et al., 1989). There is an assumption that trace metals in otoliths are deposited in proportion to dissolved concentrations in the ambient environment, while it is quite controversial (Kalish, 1991; Farrell and Campana, 1996; Thorrold et al., 1997a; Campana, 1999). Previous authors proposed that otolith aragonite is crystallized from fluid within the endolymphatic canal of the inner ear and some trace metal ions in the endolymphatic fluid are derived primarily from the ambient water (Farrell and Campana, 1996; Thorrold et al., 1997; Bath et al, 2000). However, these ions must first pass from the water into the blood plasma through the gill, and then cross another membrane into the endolymph, so some others thought the physiological changes are associated with changes in the chemistry of the blood plasma and the endolymph, which can result in differences in the trace element composition of otolith material. And these physiological changes may further complicate any correlation between water and otolith chemistry (Kalish, 1989, 1991). During the formation of otoliths, trace metals are incorporation into otoliths (Campana, 1999); the concentrations of these trace metals are thought to be influenced primarily by the environmental exposure of fish (Bath et al., 2000; Ranaldi and Gagnon, 2010). The positive relationship between the Sr:Ca and Ba:Ca ratios in the otolith and the water was demonstrated with the spot ***Leiostomus xanthurus*** (Bath et al., 2000). Ranaldi and Gagnon (2010) conducted experiments with pink snapper (***Pagrus auratus***) and proposed that otolith metal concentrations (Zn, Cd and Pb) may be related to the environmental exposure history of fish to contamination. Otoliths have the potential to record the historic exposure of fish to metal contamination and it is possible to apply otoliths as an indicator of environmental pollution (Saquet et al., 2002; Ranaldi and Gagnon, 2008, 2010; Li et al., 2011a, b; Du et al., 2011).

There are still some problems in the previous studies, such as, it is still unclear which factor affects occurrence of vateritic lapillus and vateritic sagitta of wild carps, and it is unknown, which elements can act as water chemistry monitors in a specific site. In this work, we aimed at solving the following challenges: 1. Is the mineral polymorph of otoliths linked to ambient water quality? 2. Do the trace elements in lapilli correlate to the corresponding element in water? 3. Are trace metals in otoliths deposited in proportion to dissolved concentrations in the ambient environment? 4. Which elements can serve as environmental monitors?

MATERIALS AND METHODS

Sampling location

To solve the problems mentioned earlier, two extreme quality waters were chosen (Miyun Water Reservoir with high water quality which is the drinking water source of Beijing and Baiyangdian lake which is used for agricultural water and has been polluted seriously for the past ten years (Yang et al., 2005; Luo, 2006) (Figure 1). According to the heavy metal element standard for ground water, Miyun Water Reservoir as drinking water has been keeping the second level throughout 50 years (data from Beijing water authority), whereas Baiyangdian lake has been worse than the fifth level (data from environmental condition report of China). In general, the concentration of heavy metals in Baiyangdian Lake is higher than that in Miyun Water Reservoir.

Water trace elements such as Cr, Mn, Cu, Zn, Pb, etc were determined by using inductive coupling plasma mass spectrometry (ICP-MS) by Luo et al. (2006), and a specific content comparison of trace elements from Baiyangdian lake and Miyun Water Reservoir is presented in Figure 2. Heavy metal content was logarithmically transformed because Cr and Mn content is higher than the content of Cu, Zn and Pb by nearly two orders of magnitude. From the figure, it was shown that the concentration of Cr, Mn, Cu, Zn and Pb in Baiyangdian Lake is higher than those in Miyun Water Reservoir.

Otolith preparation

A total of 38 carps were collected in September and October, 2008 from the two sites. To extract otoliths, a pair of lapilli, a pair of sagittae and a pair of asterisci were dug out from utricular, saccular and lagena, respectively. All the otoliths were dipped in ethanol of 75% for 48 h to remove adhering tissues, and then rinsed with deionized water and air dried. The fish age was 2 to 3 years old which was estimated based on the annual growth line (Figure 3). Three kinds of otoliths (6 sagittae, 9 lapilli and 7 asterisci in Baiyangdian Lake and 7 sagittae, 7 lapilli and 7 asterisci in Miyun Reservoir) from two sites were grinded into fine calcium carbonate powder with agate mortar for phase analysis. Three lapilli from each site were mounted on the slices and grinded into sections for elemental concentration analysis. The reason why sagitta and asteriscus was not chosen is that the size and shape of sagitta and asteriscus is not suitable to be sectioned. Of the three otoliths, the size and shape are species specific. For carps, the lapillus is big and thick enough to be sectioned.

Mineral phase and geochemical analysis

The calcium carbonate power was examined using X-ray powder diffractometer to identify the crystalline phases and whether or not otoliths' polymorph was linked to ambient water quality was further determined.

Six lapilli's sections were analyzed using an excimer 193 nm Laser Ablation system (GeoLas 200M, MicroLas, Göttingen, Germany) combined with an Elan 6100 DRC-ICP-MS (PerkinElmer/

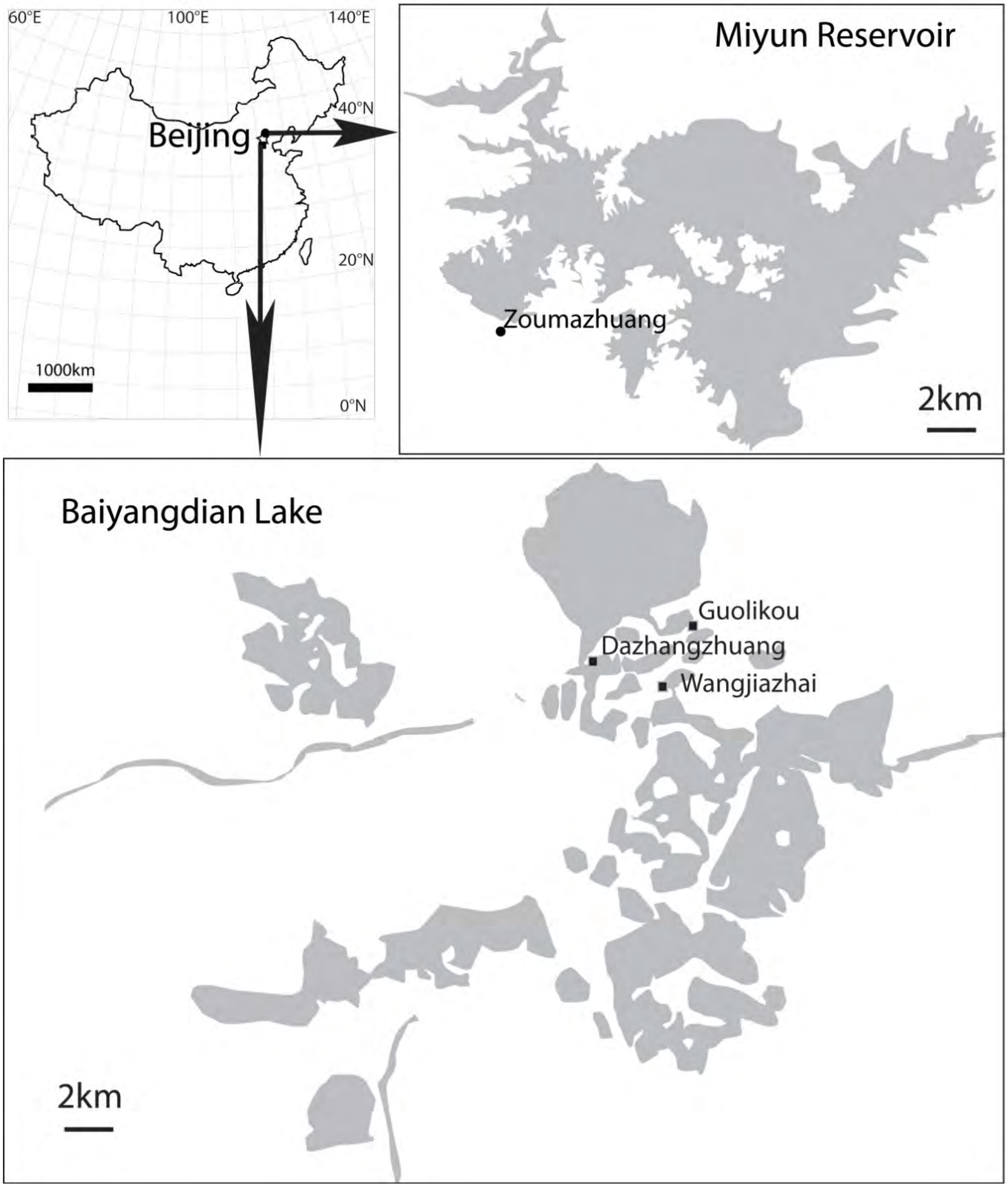

Figure 1. Map of Miyun Water Reservoir and Baiyangdian Lake and sampling locations.

SCIEX, Canada) in the LA-ICP-MS laboratory at Northwest University of China (Figure 3). A laser beam diameter of ~60 m was pulsed at a frequency of 10 Hz. Calibration was achieved using external standard with internal standardization. Ca was used as an internal standard to compensate for signal variation caused by differences in mass of ablated material. Assuming the Ca concentration to be constant at 396,000 μg/g, the concentrations of the elements were determined against this using the response factor to the known concentration in the NIST glass standard 610. The detection limit of this method for trace element was mg/kg (ppm).

RESULTS AND DISCUSSION

Mineral polymorph

Mineral polymorph of otoliths from the two sites was

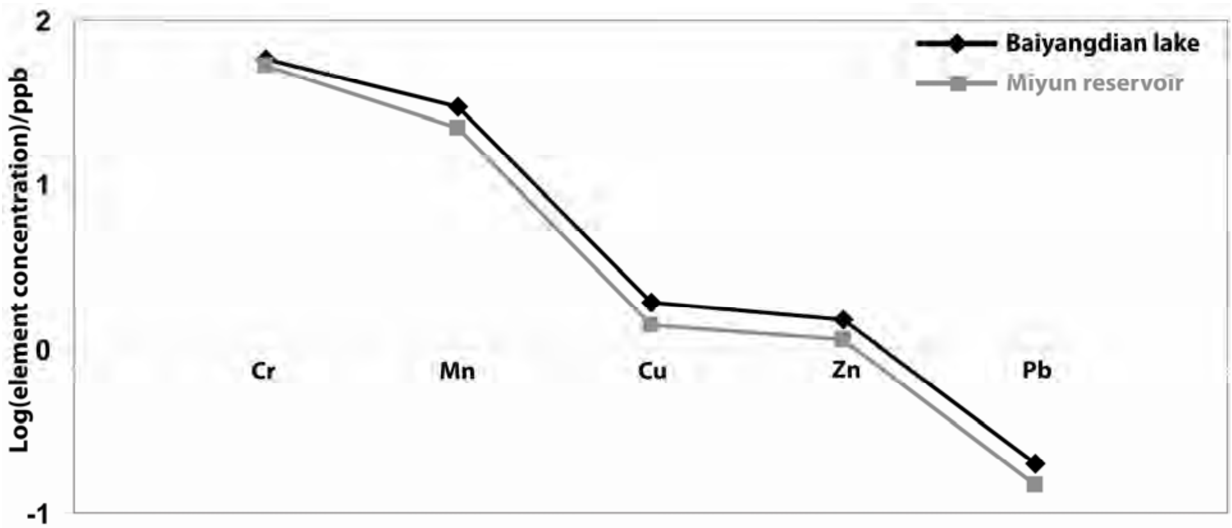

Figure 2. Comparison of specific heavy metal content between Baiyangdian Lake and Miyun Water Reservoir (Y-axis data was logarithmically transformed because Cr and Mn content are higher than the content of Cu, Zn and Pb by nearly two orders of magnitude).

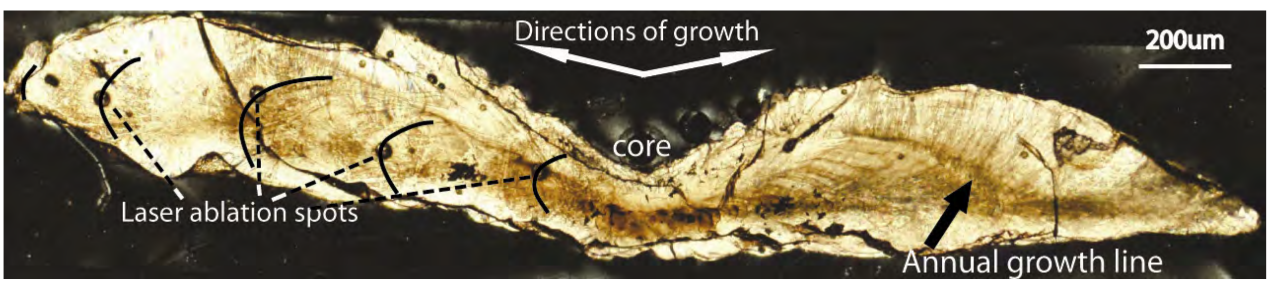

Figure 3. An example of lapillus section after laser ablation, the dashed lines point to the laser ablation spots, and the black curves show the growth line. A total of 38 laser ablation spots were obtained.

analyzed. The X-ray diffraction patterns exhibiting lapilli and sagittae were composed of aragonite, except for one specimen B-22 from Baiyangdian Lake; asterisci are made of vaterite (Figure 4). Lapillus of B-22 is matched to vaterite pattern instead of aragonite pattern, while most peaks of sagitta are matched to aragonite pattern and a few peaks are matched to vaterite pattern, as shown in Figure 5. Previous studies have shown $CaCO_3$ polymorphs differences in these three kinds of otoliths (Degens et al., 1969; Gauldie, 1993; Motta et al., 2009; Li and Feng, 2007). Most authors proposed that lapillus and sagitta are composed of aragonite, whereas asteriscus consists of vaterite (Motta et al., 2009; Li and Feng, 2007). However, the occurrence of vateritic lapillus and sigatta were also found in some fishes (Sweeting et al., 2004; Yang et al., 2007; Jessop, 2008), in which vateritic sagitta were found in cultured coho salmon and the authors supported that it was correlated with stress factors such as disease, poor water quality and temperature fluctuation (Sweeting et al., 2004). In addition, Ma et al. (2009) thought that the abnormal otolith formation (vaterite otoliths) may be related to the environmental conditions such as temperature, salinity, river discharge, pollution and biological activity. As for the otolith polymorph in Baiyangdian Lake, the poor water quality is at least one factor which could be responsible for replacement of the vaterite for aragonite. Baiyangdian Lake and Miyun Water Reservoir, which are both located close to Beijing, are approximately 200 km apart. The two waters have similar climate with four distinct seasons belonging to warm-temperate zone. The water temperature of Baiyangdian Lake ranges yearly from 4 to 26.5°C; the water temperature of Miyun Water Reservoir is up to 27.9°C in summer, and the water temperature under the ice is about 4°C during frozen period (Ge et al., 2003). Considering the similar temperature fluctuation of the two sites, we could rule out that temperature factor affect the occurrence of vateritic otoliths. The polluted water could account for the occurrence of vateritic otolith in Baiyangdian Lake under the assumption that all the fishes were healthy. Although all the fishes were collected live, we are not absolutely sure if the fish with vateritic

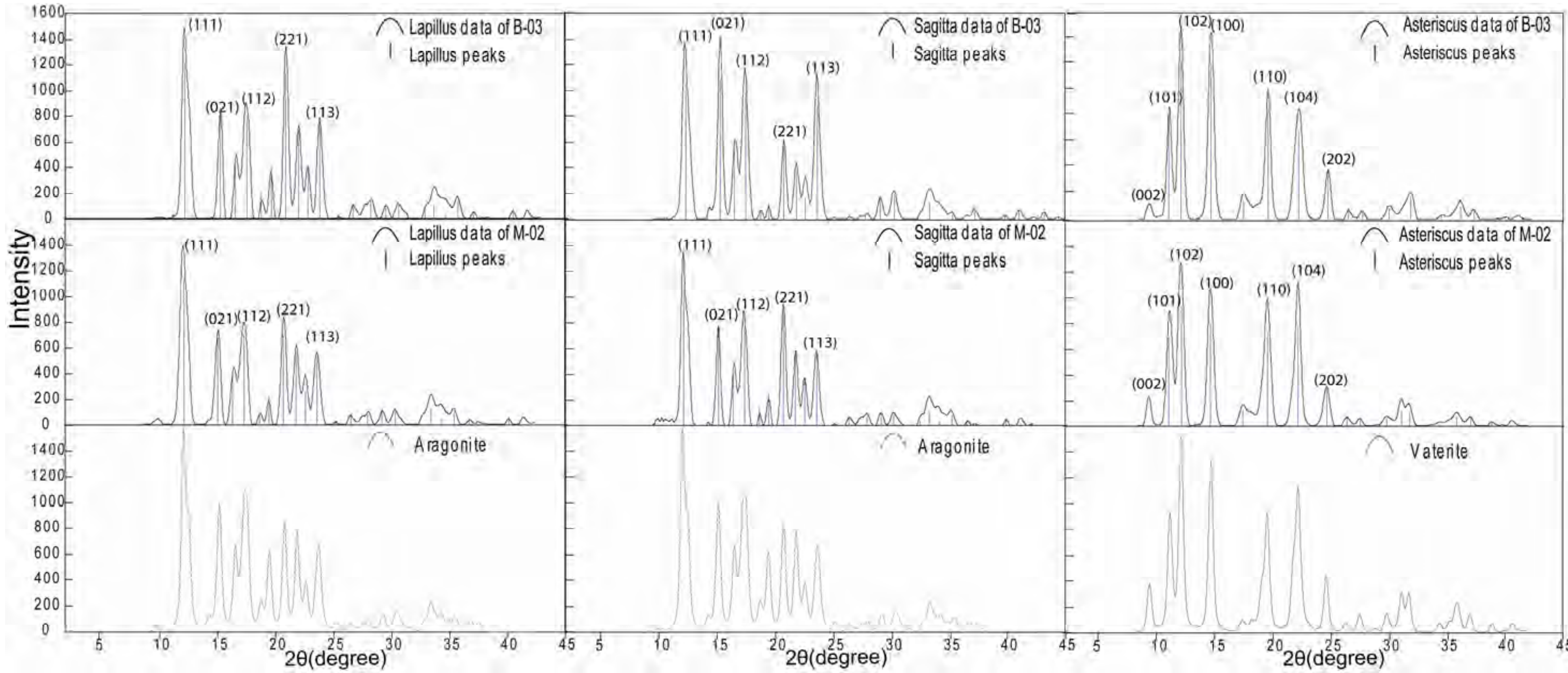

Figure 4. Examples of X-ray diffraction patterns (XRD): lapillus XRD patterns match the aragonite XRD pattern, as well as sagitta XRD pattern; asterisci are matched to the vaterite XRD pattern.(B-03 and M-02 are the IDs of fish, which are from Baiyangdian Lake and Miyun Water Reservoir, respectively).

lapillus and sagitta was without disease, however, the fishes that inhabits polluted water are more likely to get disease. Vaterite otoliths were found in Baiyangdian Lake which was polluted seriously with higher content of heavy metals. The replacement of vaterite for aragonite happened when the water environment was polluted up to a certain extent. Therefore, we are inclined to agree that the poor water quality played the greatest role for the vateritic otoliths in Baiyangdian Lake. As for the other aragonite otoliths in Baiyangdian Lake, individual difference of specimens for pollution tolerance could be one reason. As for how to determine the pollution tolerance of carps, more field specimens or cultured ones are needed for further study.

Geochemistry of otoliths

The geochemical results in each ablation spots of all otolith specimens are shown in Table 1. For the comparison of trace elemental concentration of lapilli from Baiyangdian Lake and Miyun Water Reservoir, statistical analysis was performed using SPSS statistical package 17. Mean values of each metal element (Cr, Mn, Cu, Zn and Pb) of each site were calculated, respectively. Significant difference of Pb concentration between sites was tested by ***t***-test of the compare means (***t***-test comparison: ***t*** = 2.043, P<0.05) (Figure 6), while the site-specific differences of the other metals were not significant (***t***-test comparisons: Cr: ***t*** =

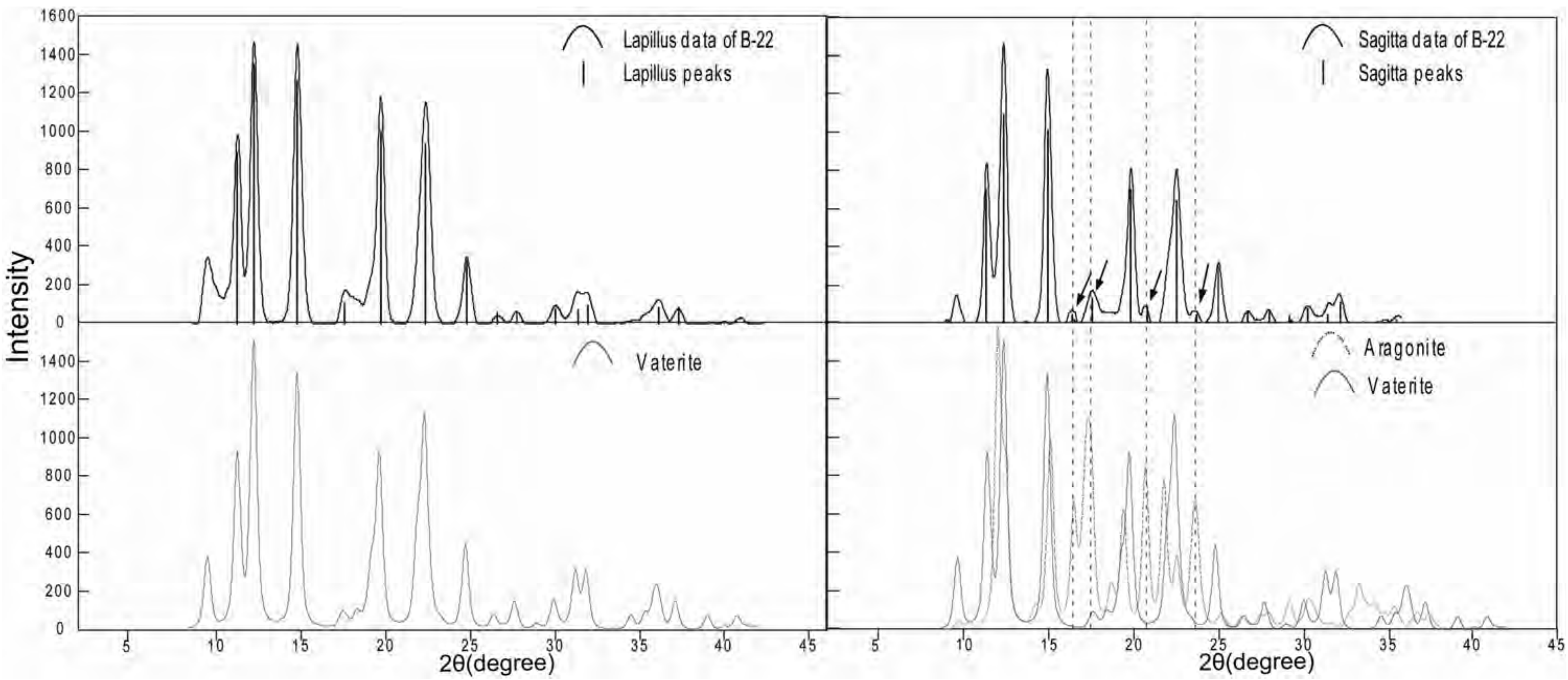

Figure 5. An exception of otolith XRD pattern, lapillus of B-22 is matched to the vaterite pattern instead of aragonite pattern, while most peaks of sagitta are matched to aragonite pattern and a few peaks are matched to vaterite pattern, as shown with the arrows (B-22 is the ID of fish, which is from Baiyangdian Lake).

1.281, P>0.05; Mn: ***t*** = 0.695, P>0.05; Cu: ***t*** = 1.364, P>0.05; Zn ***t*** = 1.527, P>0.05). In addition, a significant difference of Sn concentration was tested as well (***t***-test comparison: ***t*** = 2.652, P<0.05) (Figure 6).

The box plots provide some valuable data regarding the lapillus Pb and Sn content in Baiyangdian Lake and Miyun Water Reservoir. The mean value of lapillus Pb is 0.13 (±0.03) in Baiyangdian Lake and 0.04 (±0.01) in Miyun Water Reservoir, and the mean values of lapillus Sn are 1.01 (±0.19) and 0.16 (±0.01) in the two sites, respectively. It is apparent that average contents of lapilli Pb and Sn in Baiyangdian Lake are higher than those in Miyun Water Reservoir. In addition, average content of lapilli Pb is consistent with the water dissolved Pb measurement (Luo, 2006), with higher dissolved Pb concentration in Baiyangdian Lake, relative to the Miyun Water Reservoir (Figure 7).

According to the formula of enrichment co-efficients (otolith$_{Pb/Ca}$: water$_{Pb/Ca}$), the enrichment coefficients of each site were calculated, respectively. The results show that the coefficient of Baiyangdian Lake is 627, whereas it is 267 in Miyun Water Reservoir. Bottom sediment might be an important reason for the discrepant enrichment coefficients. The incorporation of elements into otoliths is a complex process; therefore, many factors might influence the incorporation of trace

Table 1. Metal concentrations in each ablation spots of all six otolith specimens.

Ablation spot	Cr (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Pb (ppm)
B-07-01	0.42	0.39	0.17	0.07	0.11
B-07-02	0.42	0.24	0.11	0.08	0.04
B-07-03	0.29	0.27	1.58	0.37	0.10
B-07-04	0.47	0.38	0.14	0.13	0.05
B-10-01	0.45	0.33	0.13	0.08	0.01
B-10-02	0.19	0.12	0.12	0.10	0.01
B-10-03	0.18	0.05	0.15	0.09	0.01
B-10-04	0.28	0.14	0.12	0.08	0.02
B-10-05	0.25	0.14	0.13	0.08	0.01
B-20-01	2.29	0.70	1.92	2.01	0.15
B-20-02	2.55	0.83	2.37	2.12	0.11
B-20-03	4.66	0.72	2.01	1.56	0.05
B-20-04	2.38	1.33	2.13	3.33	0.30
B-20-05	3.93	3.77	2.98	3.90	0.64
B-20-06	3.08	0.78	2.29	2.19	0.11
B-20-07	3.33	0.78	2.17	2.24	0.04
B-20-08	5.63	1.08	3.03	2.38	0.11
B-20-09	5.28	1.08	2.15	3.15	0.25
B-20-10	3.60	0.77	2.25	2.80	0.10
B-20-11	2.64	1.21	2.47	2.72	0.17
B-20-12	2.30	0.75	2.47	2.63	0.36
B-20-13	0.25	0.07	0.19	0.15	0.01
B-20-14	0.60	0.08	0.50	0.20	0.14
M-08-01	4.13	1.14	2.43	2.09	0.02
M-08-02	5.92	1.87	2.55	1.82	0.03
M-08-03	5.36	1.69	3.24	3.06	0.05
M-08-04	4.75	1.32	2.70	2.05	0.07
M-08-05	5.08	1.41	2.98	2.15	0.17
M-09-01	0.59	0.21	0.07	0.43	0.04
M-09-02	0.53	0.46	0.05	0.13	0.03
M-09-03	0.51	0.16	0.05	0.08	0.02
M-09-04	0.49	0.31	0.03	0.10	0.04
M-10-01	0.38	0.09	0.12	0.07	0.01
M-10-02	0.35	0.23	0.11	0.08	0.00
M-10-03	0.18	0.13	0.12	0.08	0.01
M-10-04	0.31	0.18	0.11	0.08	0.04

The specimens starting with B are from Baiyangdian lake, while those starting with M are from Miyun reservoir.

elements into lapillus. Because carps prefer to scavenge insects and worms from bottom sediment, food could affect the content of trace elements in otolith as well. High Pb concentration of bottom sediment in Baiyangdian Lake has been reported (Yang et al., 2005), therefore, bottom sediment with high Pb concentration may account for the greater enrichment of lapilli Pb in Baiyangdian Lake. The Pb in water and in bottom sediment was the two main sources of lapilli Pb, and it is possible for lapilli Pb to act as a monitor of water Pb, although it may be nonlinear relationship. There is limited knowledge on dissolved Sn content in the two study sites, water dissolved Sn is possibly one of the factors, therefore more data about dissolved Sn content in water is still needed to work out whether lapillus Sn can act as an environmental monitor.

Conclusion

Mineral polymorph and trace elements of carp otoliths were tested. It was found that crystalline phases of otoliths are related to water quality of ambient environment. Generally, lapilli and sagittae are composed of

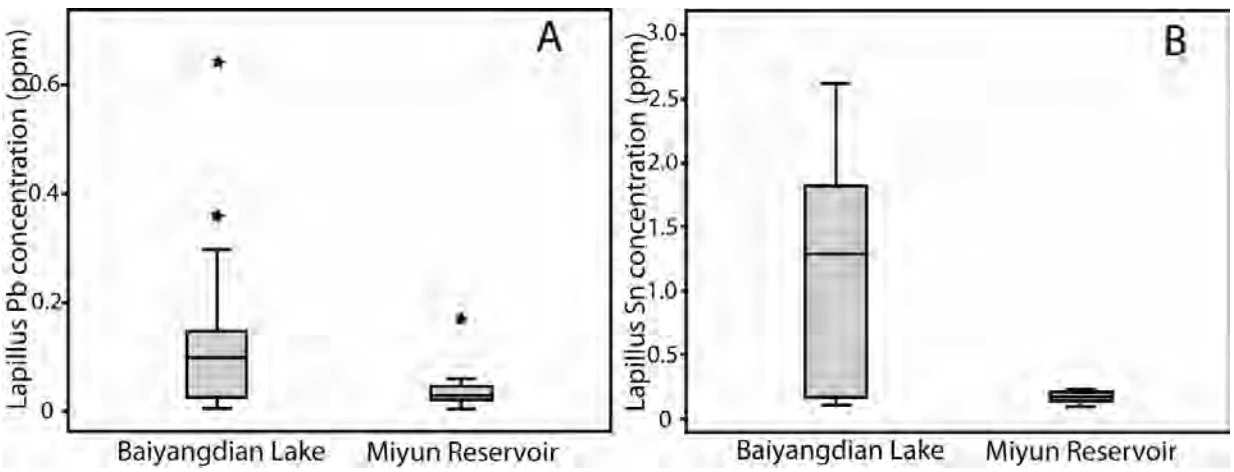

Figure 6. Mean Pb and Sn lapillus concentration of carp otoliths from fish collected from Baiyangdian Lake and Miyun Water Reservoir. Means are both significantly higher in Baiyangdian Lake than in Miyun Water Reservoir. A: Comparison of lapillus Pb concentration between two waters, standard deviation of 0.15 and 0.04 (filled stars indicate the outliers.); B: Comparison of lapillus Sn concentration between two waters, standard deviation of 0.90 and 0.03.

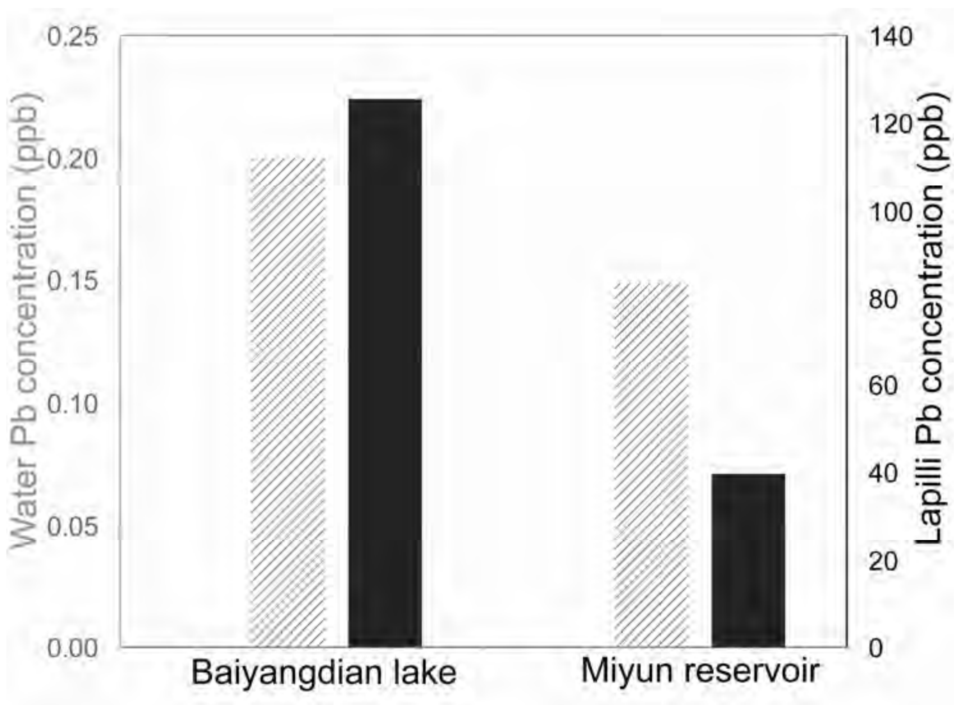

Figure 7. Histogram of mean Pb content in sites and in lapilli relatively. The grey diagonal columns indicate water Pb concentration in Baiyangdian Lake and Miyun Water Reservoir using the left scale, and the black ones depict lapillus Pb concentration in two sites using the right scale.

aragonite, and asterisci are composed of vaterite. However, the occurrence of vateritic lapillus and vateritic saggita was found in Baiyangdian Lake with poor water quality. In other words, the replacement of vaterite with aragonite happened when the water environment was polluted up to a certain extent. Therefore, we are inclined to agree that the poor water quality played the greatest role in the vateritic otoliths in Baiyangdian Lake.

Significant difference of Pb concentration in otoliths were found between Baiyangdian Lake and Miyun Water Reservoir using ***t***-test of the compared means, as well as significant difference of Sn concentration. Trace element analyses may provide trace element compositions of inhabited environment. Average content of lapilli Pb is consistent with the water dissolved Pb measurement, with higher dissolved Pb concentration in Baiyangdian Lake, relative to the Miyun Water Reservoir. Bottom sediment may account for the significant discrepancy of enrichment coefficients between sites. Otoliths proxy is a potential record of ambient chemical properties of aquatic environment, and lapilli Pb could serve as an environmental monitor, although it is nonlinear relationship.

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