

## **Mass Mortality and Trace Element Residues in Isaza (*Gymnogobius isaza*) Collected from Lake Biwa, Japan**

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**Abstract**—Isaza (*Gymnogobius isaza*) is a fish species endemic to Lake Biwa, Japan. Mass mortality of Isaza occurred in Lake Biwa during December 2007, with more than 1,800 dead specimens observed. This phenomenon was found for the first time since the year 2000 when regular observation of the lake bottom by autonomous underwater vehicle “Tantan” was started. In the present study, concentrations of 23 elements in Isaza specimens from the mass mortality event (MMS) were measured and compared with the levels in normal fish samples (NFS) collected from Lake Biwa. While mean Se, Rb, Cs and Hg levels were significantly higher in NFS than in MMS, mean V, Cr, Mn, Co, Zn, Ga, As, Sr, Mo, Cd, In, Sn, Sb, Ba, Tl and Pb concentrations were significantly higher in MMS than in NFS. Particularly, the mean levels of Mn and As were notably higher in MMS than in NFS. These results suggest that exposure to Mn and As as well as low levels of dissolved oxygen might be the reasons for the mass mortality of Isaza.

**Keywords:** Isaza, mass mortality, arsenic, manganese, hypoxia

### INTRODUCTION

Lake Biwa located in the middle of Shiga Prefecture, Japan is a rift lake formed about 400 thousand years ago and is the biggest lake in Japan. The lake is rich in biodiversity with 491 species of flora and 595 species of fauna. The lake area, which is about 674 m<sup>2</sup>, is separated into Northern and Southern basins. The depth of the water is about 104 m at most places. The amount of water in the lake is about 275 billion m<sup>3</sup>, and it equals about 30% of the available freshwater in Japan. Lake Biwa is the source of water supply for 14 million people living in Kinki area of Japan. In recent years, some abnormal phenomena such as decrease of snowfall, release of methane gas from the lake bottom, acceleration of eutrophication, rise in water temperature and reduction of dissolved oxygen (DO) level were observed in Lake Biwa. Such events are considered to be linked with global warming.

In December 2007, mass mortality of Isaza (*Gymnogobius isaza*) and lake

prawn (*Palaemon paucidens*) occurred in Lake Biwa. Isaza is a fish species endemic to Lake Biwa. Lake Biwa Environmental Research Institute estimated the number of mortalities at over 100,000 for each species. This phenomenon was observed for the first time since the year 2000 when regular observation of the lake bottom by autonomous underwater vehicle "Tantan" was started. Normally, DO of 3.0~5.0 mg/L is needed for fish to survive; however, at the time of mass mortality DO at the bottom of the lake was only 0.57 mg/L. Therefore, hypoxia assume that the one of the reason of the mass mortality of Isaza.

There are some studies on trace element levels in Lake Biwa, which have reported high levels of Mn and As in the sediments of the lake, especially in the Northern basin (Takamatsu *et al.*, 1985; Li *et al.*, 2007). Mean As level in the sediment samples from the Northern basin of Lake Biwa was 68.9 mg kg<sup>-1</sup> DW (Li *et al.*, 2007), which was higher than the levels reported for a lake in Florida, where organic As herbicides had been applied (Whitmore *et al.*, 2008), and the levels observed for a borehole in Shanxi Province of China, one of the most severely arsenic affected areas in China (Fan *et al.*, 2008).

Arsenic is usually more mobile under reducing conditions than under oxidizing conditions because of the strong tendency of elution, and hence be immobilized by, Fe and Mn oxyhydroxides, which persist only under oxidizing conditions (Kneebone and Hering, 2000). Arsenic is a toxic metalloid and may occur either as inorganic compounds or as organic As and in natural waters, As is mostly found in inorganic forms as oxyanions of As(III) or As(V) (Pedersen *et al.*, 2006). The toxicity of As compounds is markedly dependent on their chemical structure, and some methylated As compounds have lower toxicities than those of inorganic As compounds (Nakamura *et al.*, 2008).

The present study examined whether elements, especially Mn and As, were related to the mass mortality of Isaza. The reasons to suspect involvement of Mn and As in the mass mortality were as follows: 1) High levels of Mn and As in the sediments, 2) Low DO level at the bottom, and 3) Bottom dwelling nature of Isaza.

To clarify the hypothesis, concentrations of the trace elements and speciation and levels of As compounds in normal alive and dead specimens of Isaza were compared.

## MATERIALS AND METHODS

### *Samples*

Ten specimens of Isaza (*Gymnogobius isaza*) from the mass mortality event (MMS) were collected in December 2007 and 5 normal fish samples (NFS) were caught in February 2008. Six MMS and 3 NFS samples were analyzed for As speciation. About 200 g of normal specimens of rosy bitterling (*Palaemon paucidens*) were divided into 3 sub-samples. Twenty dead specimens (*ca.* 5 g) of rosy bitterling were pooled into one sub-sample. Whole body samples of Isaza and sub-samples of rosy bitterling were freeze-dried and uniformly homogenized.

## Chemical analyses

### Trace element analysis

Concentrations of 22 elements (Mg, V, Cr, Mn, Co, Ni, Cu, Zn, Se, Rb, Sr, Mo, Ag, Cd, In, Sn, Sb, Cs, Ba, Tl, Pb and Bi) were determined by ICP-MS (Hewlett-Packard, HP-4500). Yttrium was used as an internal standard for ICP-MS measurements. Mercury concentration was determined with a cold vapor AAS (CV-AAS) (Sanso, Model HG-3000). Total As analysis was conducted following the method of Kubota *et al.* (2001) with a slight modification. About 0.05 g of freeze-dried tissue was digested with acid mixture ( $\text{HNO}_3:\text{H}_2\text{SO}_4:\text{HClO}_4 = 5\text{ml}:5\text{ml}:10\text{ml}$ ) by heating. Total As concentration was measured by a hydride generation (HVG-1 Hydride System, Shimadzu, Kyoto, Japan)—atomic absorption spectrometry (AA680, Shimadzu, Kyoto, Japan) (HG-AAS). Accuracy of these analyses was checked by using two standard reference materials, dogfish liver (DOLT-3) provided by National Research Council Canada (NRCC) and oyster tissue (1566b) provided by National Institute of Standards and Technology (NIST). The recovery rates of this procedure ranged from 95.2% (Rb) to 103.6% (Sb) for measured element concentrations. The recovery rate of As was 100% using both standard reference materials.

### Analysis of As compounds

Arsenic compounds were extracted by methanol/Milli-Q water (9:1 v/v) from powdered freeze-dried samples (*ca.* 0.15 g) (Kubota *et al.*, 2002). Supernatant was concentrated until methanol was evaporated. After evaporation, samples were diluted by Milli-Q water and then filtered by a 0.45  $\mu\text{m}$  filter. Arsenic that was remaining in the sample after extraction (residue As) was measured by HG-AAS after acid digestion mentioned above. A Hamilton PRPX100 anion-exchange column (Hamilton, Reno, NV, USA;  $\text{NH}_4\text{H}_2\text{PO}_4$  buffer, pH 6.0) and a Supelcosil LCSCX cation-exchange column (Supelco, Bellefonte, PA, USA; pyridine buffer, pH 2.6) were used to separate the As compounds. As internal standards, Rh were added to both mobile phases to monitor analytical interferences. Eight As compounds (AB, AC, TETRA, TMAO, DMA, MMA, As[III], and As[V]) were measured by a high-performance liquid chromatography (HPLC; Shimadzu, LC10A Series, Kyoto, Japan)/inductively coupled plasma mass spectrometry (ICP-MS; HP4500, Hewlett-Packard, Avondale, PA, USA) (HPLC/ICP-MS). The accuracy of As compound concentrations in this study was confirmed with a standard reference material of tuna fish muscle (BCR-627; the European Commission DG Joint Research Centre, Institute for Reference Materials and Measurements). In the present study, the concentrations of As compounds are presented in units of  $\mu\text{g As/g dry wt}$ . The sum of eight As species and residual As are shown as RAs.

### Statistical analysis

Correlations between the metal concentrations in the samples were examined using the Spearman rank test. The statistical differences in concentrations between MMS and NFS were analyzed using the Mann-Whitney *U*-test. A *p*-

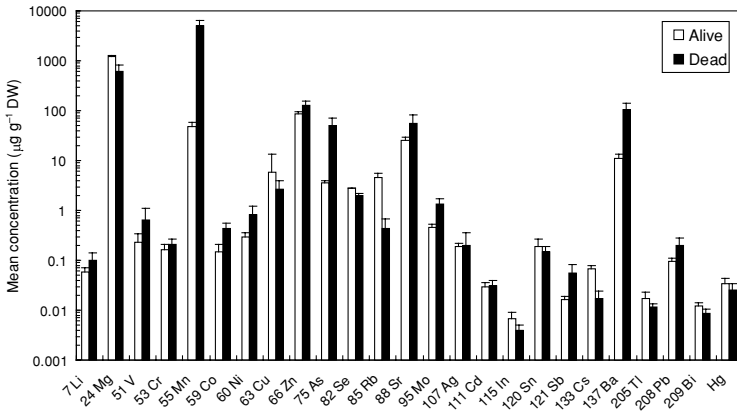


Fig. 1. Comparison of mean element concentrations between NFS and MMS.

value  $< 0.05$  was considered statistically significant. All of the statistical analyses were executed by the Statcel2 program (Yanai, 2004).

## RESULTS AND DISCUSSION

Trace element concentrations in NFS and MMS are shown in Fig. 1. While mean Mg, Se, Rb, In, Cs, Tl and Bi levels were significantly higher in NFS than in MMS, mean Li, V, Cr, Mn, Co, Ni, Zn, As, Sr, Mo, Sb, Ba and Pb concentrations were significantly higher in MMS than in NFS. Particularly, the mean levels of Mn and As were notably higher in MMS than in NFS.

Mean values of Mn in NFS and MMS were  $48.9 \mu\text{g g}^{-1}$  DW and  $5080 \mu\text{g g}^{-1}$  DW, respectively. Mean values of total As in NFS and MMS were  $3.62 \mu\text{g g}^{-1}$  DW and  $51.1 \mu\text{g g}^{-1}$  DW, respectively. Generally, the As concentrations found in MMS were higher than in the freshwater fish from Zagreb (mean value;  $23.5 \pm 36 \mu\text{g g}^{-1}$  DW) (Bošnjir *et al.*, 2003) and from Hong Kong (maximum value;  $3.44 \pm 0.40 \mu\text{g g}^{-1}$  WW) (Cheung *et al.*, 2008), places known to have serious As pollution (Fig. 2).

While the mean As levels in both NFS and MMS were in the range observed in marine organisms, they were much higher than in general freshwater organisms. Toxicity of As depends on its chemical speciation. Many marine organisms are known to accumulate As mostly as aqueous-organic As compounds, which are much less toxic than inorganic As compounds. Therefore, in order to clarify whether As toxicity is related to the mass mortality of *Isaza*, chemical speciation analysis of As should be performed.

Concentrations of As chemical species in *Isaza* and rosy bitterling are shown in Table 1. Kinds of As species detected *Isaza* of MMS than the NFS. Inorganic As(III) which is one of the most toxic As compounds was detected in all samples of MMS and dead RB, and the proportions of this chemical species in total As

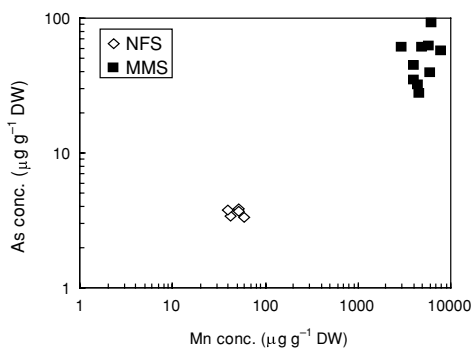


Fig. 2. Relationship between Mn and As concentrations in NFS and MMS.

were 20~50% in MMS. This fraction of As was also higher in MMS and dead RB than in NFS and fresh RB. On the other hand, AB, which is a less toxic form, was not detected in NFS, MMS and RB. Whereas kinds of species detected Isaza of MMS than the NFS, inorganic As(III), which is one of the most toxic As compounds, was detected in both MMS and NFS as well as rosy bitterling. Interestingly, the proportion of this chemical species to total As was higher in NFS than in MMS. These results imply that expression of As toxicity may not contribute to the proportion of As(III) to T-As but to the concentration.

In the aquatic ecosystems like Lake Biwa, there are various As species because of mediation of phytoplankton in the oxidized layer. Methyl As and As(III) compounds transformed by phytoplankton are decomposed into As(V). In Lake Biwa, a part of As(V) is adsorbed on the particles of Fe–Mn oxide and sediments and settles to the bottom of the lake. Another part of As(V) is assimilated by phytoplankton.

On the other hand, in anoxic layer, dissolution of As(V) and As(III) from the sediment is accelerated. Methyl As compounds transformed by phytoplankton are decomposed into As(V) and As(III). The variation of As chemical species is simpler in anoxic layer than in oxidized layer. Inorganic As species such as As(V) and As(III) are dominant in anoxic layer. There were fewer kinds of As species in MMS than in NFS. This may be reflected by the behavior of As compounds in anoxic layer at the bottom of Lake Biwa.

In the present study, inorganic As, both As(III) and As(V) were detected in Isaza and rosy bitterling. The chemical forms of As detected in MMS were fewer than in NFS. Concentrations of aquatic residues of As were relatively higher in MMS and dead rosy bitterling than in NFS and fresh rosy bitterling.

To further elucidate whether mass mortality of Isaza was caused by the exposure of dissolved As(III) from the bottom of lake Biwa, we need 1) to understand the redox status and the behavior of As and Mn at the bottom of Lake Biwa, 2) to carry out continuous monitoring of trace elements, and 3) assay toxic effects of As and Mn on Isaza.

Table 1. Concentrations of As chemical species in Isaza and rosy bitterling.

Sample	T-As	As(V)	As(III)	MMA	DMA	AB	TMAO	TETRA	AC	SUM	Water	Lipid	Residue	Unknown peak	SUM+Lip+Res	Recovery (%)
Isaza A-1	3.89	0.438	0.818	0.170	0.1199	—	0.459	—	—	2.01	1.64	0.361	1.21	2	3.57	91.9
Isaza A-2	3.70	0.333	1.00	0.123	0.0685	—	0.641	—	—	2.16	1.66	0.345	1.52	3	4.03	109
Isaza A-3	3.42	0.167	0.360	0.129	0.091	—	0.501	—	—	1.25	1.34	0.253	1.14	2	2.64	77.2
Isaza D-1	61.4	14.8	18.2	—	—	—	0.209	—	—	33.3	27.9	6.00	26.9	—	66.2	108
Isaza D-4	39.0	7.53	4.31	—	—	—	0.391	—	—	12.2	8.75	4.63	17.8	—	34.7	88.8
Isaza D-5	44.7	8.06	4.32	—	—	—	0.210	—	—	12.6	10.7	5.22	18.6	—	36.5	81.6
Isaza D-6	27.6	5.44	2.64	—	—	—	0.433	—	—	8.51	7.12	3.55	13.4	—	25.5	92.1
Isaza D-8	61.2	14.1	5.25	—	0.0928	—	0.205	—	—	19.6	11.0	5.85	26.2	—	51.7	84.5
Isaza D-10	91.5	16.1	15.3	—	—	—	0.187	—	—	31.6	25.7	6.29	46.3	—	84.2	92.0
RB A-1	5.72	0.863	0.813	—	0.0787	—	—	—	—	1.75	2.70	0.367	2.36	2	4.48	78.4
RB D-1	—	0.673	3.36	—	0.2781	—	0.155	—	—	4.47	3.76	0.271	21.4	1	26.1	—

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