

## Fluctuations of nitrogen isotope ratio of gobiid fish (*Isaza*) specimens and sediments in Lake Biwa, Japan, during the 20th century

**Abstract**—Nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) of sediment and formalin-fixed fish specimens (*Isaza* fish, *Chaenogobius isaza*, Tanaka), which had been collected and preserved over 40 yr, were used to provide a new methodology to reconstruct recent eutrophication and historical changes in the nitrogen cycle in Lake Biwa.  $\delta^{15}\text{N}$  records of *Isaza* fish collected in the north basin of Lake Biwa, a lake that had been rapidly eutrophicated in the last several decades, showed a rapid increase from the early 1960s to 1980s with an overall amplitude of  $>3\%$ . The same trend was simultaneously observed from that of sedimentary nitrogen. We evaluated several factors controlling  $\delta^{15}\text{N}$  of *Isaza* fish and sediments during the 20th century and concluded that an increase of  $\delta^{15}\text{N}$  was either associated with an increase in loading nitrate with high  $\delta^{15}\text{N}$  values from the watershed or enhanced denitrification in the lake.

Intensified anthropogenic activities during the 20th century have accelerated eutrophication in many lakes located in suburban areas. For example, since the 19th century, anthropogenically induced eutrophication has been widely recognized in the Great Lakes of the United States and Canada, in which numerous attempts have been made to precisely describe the phenomena (e.g. Stevens and Neilson 1987; Hodell and Schelske 1998). To fully understand the mechanisms and processes of this “cultural” eutrophication, we have to trace the historical variations of lake water quality, including nutrient loading and biogeochemical processes prevailing in the lake, with sufficient time resolution. However, direct monitoring of the lake water quality for the last several decades has been done in only a limited number of lakes. Therefore, it is necessary to establish new approaches and media for reconstructing the past lacustrine environments with a sufficient time resolution and a high sensitivity.

The stable isotope ratio of nitrogen ( $\delta^{15}\text{N}$ ) could be a promising proxy for delineating the eutrophication in the lake. Nitrogen is not only one of the important nutrient elements in a lake, but it is also abundant in anthropogenic sewage and chemical fertilizers such as ammonium sulfate. Furthermore, a range in fractionations of nitrogen isotope ratios in lake processes make nitrogen isotope ratios an excellent tracer to monitor eutrophication (e.g., Cabana and Rasmussen 1996).

In the present study, we measured nitrogen isotope ratios of muscular tissue of formalin-fixed fish specimens (Gobiid fish, *Isaza*), which had been collected since 1916 from Lake Biwa, Japan. In Lake Biwa, eutrophication proceeded during the last four decades because of anthropogenic perturbations such as increased domestic sewage and reduced macrophyte area, which plays an important role in water purification (Tezuka 1992; Nakanishi and Sekino 1996). At present, the trophic status of the north and south basins of Lake Biwa are at mesotrophic and eutrophic, respectively (Nakanishi and

Sekino 1996; Shiga Prefecture 1951–1997). Together with nitrogen isotope ratios of a sediment core, we reconstruct the historical changes in  $\delta^{15}\text{N}$  of Lake Biwa during the 20th century and discuss the cause of the  $\delta^{15}\text{N}$  fluctuations.

Lake Biwa is the largest lake in Japan and is located in the central part of Honshu Island. The lake covers 674 km<sup>2</sup> and has a volume of  $\sim 27.5$  km<sup>3</sup> yr<sup>-1</sup> (Okuda and Kumagai 1995). The maximum depth of the lake is 104 m. The lake is divided into the north and south basins on the basis of topographic features (Fig. 1). Many rivers flow into the lake, whereas only one river flows out, the Seta River located at the southern tip of the south basin. The residence time of lake water has been estimated to be 14.5 yr (Okamoto 1984). Lake Biwa has undergone large changes in nitrate concentration during the 20th century as a result of elevated nitrate loading from the surrounding watershed (Nakanishi and Sekino 1996; Shiga Prefecture 1951–1997). Monthly measurements of mean concentrations of dissolved nitrate in the north basin indicated an increase from 2.7  $\mu\text{M}$  (in the 1950s) to 9.0  $\mu\text{M}$  (in the 1990s) in the epilimnetic and 2.7  $\mu\text{M}$  (in the 1950s) to 18.7  $\mu\text{M}$  (in 1990s) in the hypolimnetic waters (Shiga Prefecture 1951–1997). In this study we focused on the north basin, which holds 98.9% of lake water. Because Lake Biwa is a warm monomictic lake, the water in the north basin is well mixed from January to March, whereas the lake undergoes thermal stratification and forms a seasonal thermocline from April to December. Mean chlorophyll *a* concentrations from 1989 to 1995 showed bimodal peaks in May (5.2  $\mu\text{g L}^{-1}$ ) and October (4.9  $\mu\text{g L}^{-1}$ ) in the epilimnion at the central part of the north basin (Shiga Prefecture 1992–1997).

The Gobiid fish *Isaza* (*Chaenogobius isaza*, Tanaka) lives in water deeper than 30 m during the day and comes up close to the surface to feed during the night (Nagoshi 1982). From early April to late June, they migrate to shallow gravelled areas to spawn (Nagoshi 1966). Most individuals spawn and die in the second year. According to Nagoshi and Kojima (1976) the mean standard length (the length from top part of snout to base part of caudal fin) of the first and the second year fish are 3.6 and 5.9 cm, respectively, and the minimum body length of mature *Isaza* is  $\sim 4$  cm. The main diet of *Isaza* fish consists of gammarids (*Anisogammarus annandalei*) and zooplankton (*Daphnia galeata*, *Diaphanosoma brachyurum*, *Mesocyclops leuckarti*, and *Leptadora kindtii*) that are part of the pelagic food chain of Lake Biwa, which is based on phytoplankton (Nakanishi and Nagoshi 1984).

**Sample collection and analyses**—*Isaza* fish were caught by use of a *Isaza*-biki trawl net (mesh size is 3 mm) from late November to late December in 1916, 1953, and every year from 1963 to 1995 (Nagoshi 1966). The sampling site

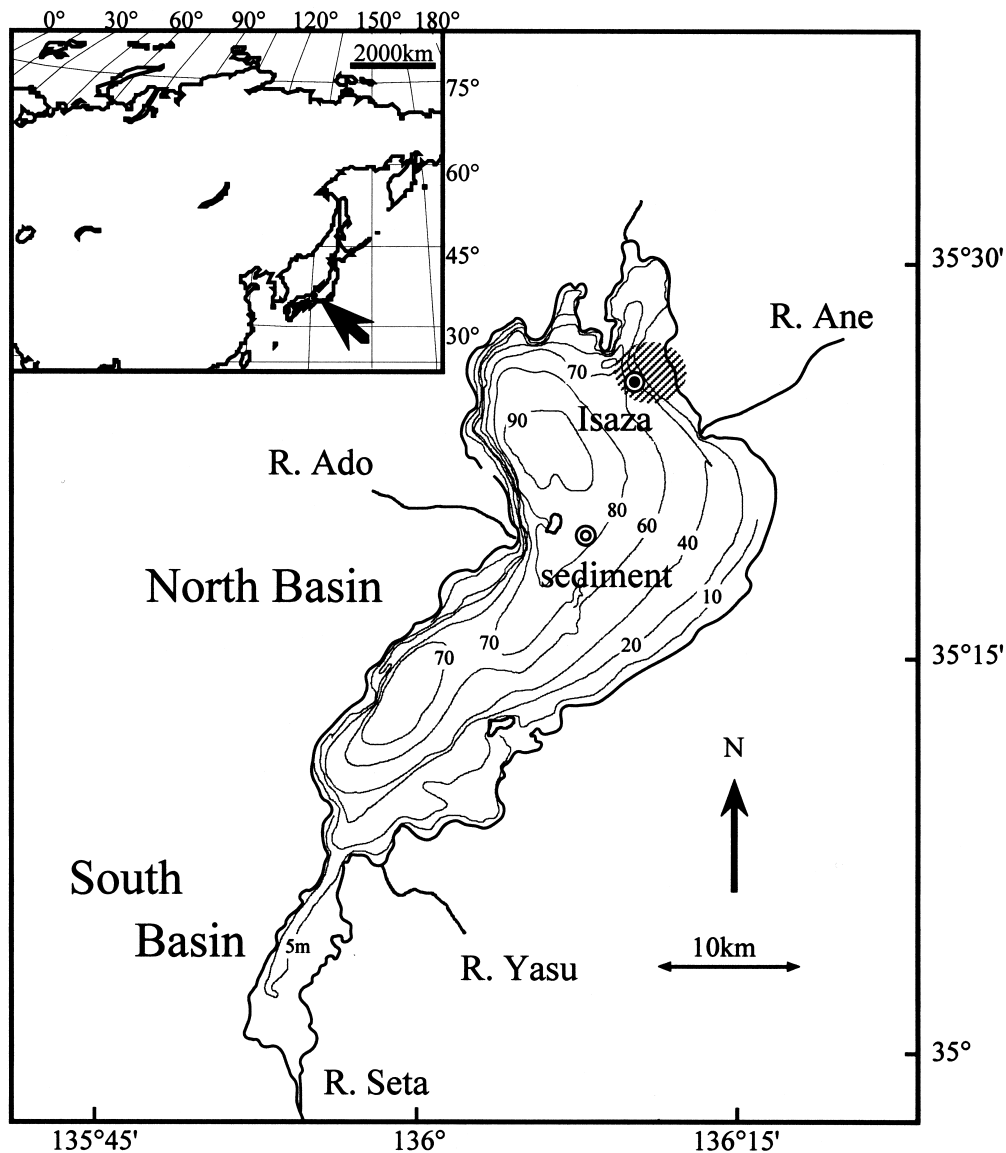


Fig. 1. Map of Lake Biwa. Isaza fish samples were collected in the northern part of the north basin (shaded area). A sediment core was recovered from the central part of the north basin.

is located offshore of the north basin (Fig. 1). Isaza fish specimens were fixed by 10% formalin, rinsed with water, and stored in 70% ethanol. In this study, the specimens 5.0–6.5 cm of standard length (probably second-year fish) were selected (Table 1). Only the muscle tissue of each specimen was subjected to chemical analysis. The muscle tissue samples were dried in the oven at 60°C for several days and crushed to a powder. The difference in  $\delta^{15}\text{N}$  between whole body samples and muscular tissue of Isaza specimens was 0.03‰ ( $n = 10$ ,  $\text{SD} = 0.6\text{‰}$ ) on average, when samples were randomly picked up from Isaza specimens collected from 1916 to 1993.

A 46-cm-long sediment core was collected from the north basin in 1995 by use of a gravity corer (Fig. 1; water depth of 85 m). The core was carefully cut in 0.5-cm increments from the core-top to 12-cm depth. The sediment samples were dried in the oven at 60°C for a few days and then

pulverized. Half of each powdered sediment sample was treated with 0.5 N HCl and rinsed with distilled water to remove carbonate carbon.

From the top down to 9.0 cm depth in the core, we selected eight sections and determined excess  $^{210}\text{Pb}$  ( $^{210}\text{Pb}_{\text{excess}}$ ) concentrations to determine the chronology of the sediment core. Approximately 1 g of dry-powdered sediment was sealed into a plastic vessel and left for at least 2 weeks in order to establish radioactive equilibrium between  $^{222}\text{Rn}$  (half life 3.8 d) and  $^{214}\text{Pb}$ . Then, specific gamma ray of  $^{210}\text{Pb}$  was monitored by a well-type pure-germanium detector (EG&G ORTEC Co., GWL-120230-S). The gamma ray spectrum was obtained by use of a multi-channel analyzer. The activity of  $^{210}\text{Pb}_{\text{excess}}$  was calculated by subtracting  $^{214}\text{Pb}$  under the assumption that  $^{214}\text{Pb}$  was in equilibrium with *in situ* decay of  $^{226}\text{Ra}$ . On the basis of the assumption that the flux of  $^{210}\text{Pb}_{\text{excess}}$  has been constant during the 20th century, a rela-

Table 1. Nitrogen isotope ratio of Isaza fish specimens collected from the north basin.

Sampling		Standard length (mm $\pm$ SD)	$\delta^{15}\text{N}$ (‰ $\pm$ SD)	<i>n</i>
Year	Date			
1916	Jan	54	12.6	1
1953	31 Jan	65	13.3	1
1963	25 Nov	53	13.9	1
1964	10 Dec	60.0 $\pm$ 1.7	14.2 $\pm$ 0.16	6
1965	Nov	57	13.0	1
1965	Dec	51	13.8	1
1967	10 Nov	62	12.9	1
1968	10 Dec	57	15.1	1
1969	05 Dec	58	15.2	1
1970	10 Dec	59.3 $\pm$ 3.2	14.4 $\pm$ 0.08	3
1971	10 Dec	60	15.5	1
1972	08 Dec	64	14.3	1
1973	10 Dec	56	15.1	1
1974	22 Dec	59	15.3	1
1975	10 Dec	57	16.0	1
1976	10 Nov	56	15.5	1
1977	10 Dec	65	15.1	1
1978	13 Dec	56	15.5	1
1979	09 Nov	56	16.1	1
1981	10 Dec	62	16.5	1
1982	10 Dec	60	15.6	1
1983	10 Dec	64	16.2	1
1984	10 Dec	58	16.1	1
1985	10 Dec	57	16.3	1
1986	10 Dec	59	16.7	1
1987	09 Dec	61	15.7	1
1988	10 Dec	60	16.1	1
1989	08 Dec	61	16.4	1
1990	13 Dec	60.5	16.2	2
1992	15 Dec	54	15.7	1
1994	25 Dec	55	15.2	1

relationship between  $^{210}\text{Pb}_{\text{excess}}$  concentration (Bq/g) and  $^{210}\text{Pb}$  age (year; A.D.) of the sediment is explained by the following equation:

$$A = -1/\lambda \ln(X_t/X_0),$$

where  $X_0$  and  $X_t$  are  $^{210}\text{Pb}_{\text{excess}}$  at initial time and at time  $t$ , respectively, and  $\lambda$  is the decay constant of  $^{210}\text{Pb}$  (0.03108  $\text{yr}^{-1}$ ).  $X_0$  is the same value of  $^{210}\text{Pb}_{\text{excess}}$  concentration at sediment-water interface and, therefore, the above equation can be expressed as follows:

$$A = -1/\lambda \ln[X_t/(\phi/S)],$$

where  $\phi$  and  $S$  are the accumulation rates of  $^{210}\text{Pb}_{\text{excess}}$  and sedimentary particles at the sediment-water interface. At a location close to our sampling site,  $\phi$  and  $S$  had been determined to be  $0.45 \pm 0.004 \text{ pCi cm}^{-2} \text{ yr}^{-1}$  (Nakamura et al. 1987) and  $0.019 \text{ g cm}^{-2} \text{ yr}^{-1}$  (Kamiyama et al. 1982), respectively. The calendar year of sediment samples was estimated to be from 1901 (at 8.5-cm depth) to 1995 (at core top) on the basis of  $^{210}\text{Pb}$  dating (Table 2). We did not find any peaks possibly related to the Chernobyl radionuclide effect. In the following discussion, we neglected bioturbation effect, because we could not identify a clear mixed layer in the sediment surface.

The  $\delta^{15}\text{N}$  values of organic substances were determined by use of the standard combustion method after Minagawa et al. (1984). Molecular sieves (Wako Chemicals, 5A 1/16) were used to collect  $\text{N}_2$  gas. Isotopic measurements of  $\text{N}_2$  gas were performed by use of Finnigan MAT Delta-S and 252 mass spectrometers. Nitrogen isotopic ratio is expressed as permil (‰) deviation from the standard (atmospheric  $\text{N}_2$ ) as defined by the following equation:

$$\delta^{15}\text{N} = [({}^{15}\text{N}/{}^{14}\text{N})_{\text{sample}}/({}^{15}\text{N}/{}^{14}\text{N})_{\text{standard}} - 1] \times 1000 \text{ (‰)}.$$

The analytical error is within  $\pm 0.2\%$ .

Table 2. Summary of analytical results of a sediment core collected from the north basin of Lake Biwa. Details on  $^{210}\text{Pb}$  age calculation are described in the text.

Sample no.	Depth (cm)	$^{210}\text{Pb}_{\text{excess}}$ (pCi $\text{g}^{-1} \pm$ SD)	$^{210}\text{Pb}$ age (yr)	$\text{d}^{15}\text{N}$ (‰, air)	Total N (mg $\text{g}^{-1}$ ds)	TOC (mg $\text{g}^{-1}$ ds)	C:N (mg $\text{g}^{-1}$ ds)
Surface 1	0–0.1	ND	1995*	8.1	5.83	51.0	10.2
Surface 2	0.1–0.2	ND	1993*	7.9	4.92	43.7	10.4
1	0.2–0.7	19.02 $\pm$ 3.05	1989	7.4	4.03	37.9	11.0
2	0.7–1.2	14.33 $\pm$ 0.61	1980	ND	ND	ND	ND
3	1.2–1.7	ND	1974*	6.4	3.64	32.9	10.5
4	1.7–2.2	10.20 $\pm$ 1.10	1969	6.1	2.27	20.3	10.5
5	2.2–2.7	6.74 $\pm$ 0.60	1956	4.9	3.33	27.5	9.6
6	2.7–3.2	ND	1952*	4.8	2.42	19.9	9.6
7	3.2–3.7	ND	1947*	4.7	2.35	19.2	9.6
8	3.7–4.2	ND	1943*	4.7	2.35	19.1	9.5
9	4.2–4.7	ND	1939*	4.7	2.27	18.7	9.6
10	4.7–5.2	ND	1935*	4.6	2.15	17.4	9.4
11	5.2–5.7	3.17 $\pm$ 0.77	1931	4.8	2.01	16.9	9.8
12	5.7–6.2	2.64 $\pm$ 0.42	1925	4.1	1.89	15.2	9.4
13	6.2–6.7	ND	1922*	3.9	1.65	12.5	8.8
14	6.7–7.2	ND	1918*	4.1	1.79	13.5	8.8
15	7.2–7.7	ND	1914*	3.9	1.66	12.4	8.7
16	7.7–8.2	1.68 $\pm$ 0.63	1911	4.2	1.65	12.9	9.1
17	8.2–8.7	1.24 $\pm$ 0.52	1901	3.8	1.75	13.3	8.9

\* Estimated by linear interpolation.

Table 3. Effect of formalin fixation to nitrogen isotope ratio of fish samples. All sample fish were collected from the south basin of Lake Biwa. Only muscular tissues were served to the experiments without defat treatment.

Species, sampling date	Standard length (cm)	Wet weight (g)	$\delta^{15}\text{N}$ (‰, air)				Average $\pm$ SD
			Period of formalin fixation (weeks)				
			0	9	62	117	
<i>Hemibarbus barbus</i>							
26 Jun 1995	16.2	62	13.9	14.0	13.9	14.0	13.9 $\pm$ 0.0
26 Jun 1995	16.4	62	14.8	14.7	15.3	14.6	14.9 $\pm$ 0.3
26 Jun 1995	18.0	90	14.1	13.8	13.9	13.7	13.9 $\pm$ 0.2
26 Jun 1995	19.2	110	13.8	13.3	ND		13.5 $\pm$ 0.3
<i>Lepomis macrochirus</i>							
26 Jun 1995	13.1	117	15.5	15.6	15.2	15.5	15.5 $\pm$ 0.2
<i>Micropterus salmoides salmoides</i>							
26 Jun 1995	16.2	108	16.9	17.0	16.7	16.6	16.8 $\pm$ 0.2
26 Jun 1995	16.9	116	ND	17.0	16.9	16.8	16.9 $\pm$ 0.1
<i>Ospariichthys uncirostris uncirostris</i>							
17 Jun 1995	12.5	30	ND	14.9	ND	15.1	15.0 $\pm$ 0.1
<i>Zacco platypus</i>							
17 Jul 1998	7.8	11	11.7	12.4	ND	12.3	12.1 $\pm$ 0.4
17 Jul 1995	14.8	63	11.9	11.8	12.2	12.2	12.0 $\pm$ 0.2

**Effect of formalin fixation**—Fixing and storing fish specimens in formalin and then in ethanol for many years could have potentially altered the  $\delta^{15}\text{N}$  of the samples and severely compromised their use as a paleo-lake proxy. In this study, we experimentally evaluated the effect of fixation and long-term storage in formalin and ethanol on the nitrogen isotopic composition of fish specimens. The muscular tissue of nine Lake Biwa fish samples was separated into five to six pieces (Table 3). One of the subsamples was immediately dried at 60°C (unfixed sample) and was used for the isotopic measurements. The remaining subsamples were soaked in 5% formalin for 9, 62, and 117 weeks (formalin-fixed samples) or in ethanol (ethanol-fixed samples) for 9 weeks before the isotopic measurements. As illustrated in Fig. 2,  $\delta^{15}\text{N}$  values were not significantly different ( $<0.2\text{‰}$ ) among the formalin-fixed, ethanol-fixed, and unfixed samples. Furthermore, the  $\delta^{15}\text{N}$  values varied little (from  $\pm 0.1\text{‰}$  to  $\pm 0.4\text{‰}$ ) throughout the period of formalin fixation (Table 3). Although the species of fish specimens used in these experiments are not Isaza, the effect of formalin showed the same results for all five species, whose trophic levels and  $\delta^{15}\text{N}$  values cover wide ranges. Lindsay (1998) also conducted similar experiments and concluded that fixing fish specimens with formalin and ethanol does not affect  $\delta^{15}\text{N}$  values of the fish (*Engraulis japonicus*) samples. Therefore, we conclude that the effect of formalin fixation and storage in ethanol on the nitrogen isotopic signature is negligible.

**Analytical results of Isaza and sediments**—Carbon contents of Isaza fish specimens showed virtually constant, with values ranging from 50.3% to 51.2% (vs. dry weight) and a mean value of 50.8%. Nitrogen content was also constant with values ranging from 13.2% to 14.8% (vs. dry weight) and a mean value of 14.1%. The C:N molar ratio was 4.2  $\pm$  0.2. The  $\delta^{15}\text{N}$  value of Isaza fish ( $\delta^{15}\text{N}_{\text{Isaza}}$ ) was 12.6‰

and 13.3‰ in 1916 and 1953, respectively, and then rapidly increased from the mid-1960s to the 1980s, although some samples exhibited rather low  $\delta^{15}\text{N}_{\text{Isaza}}$  values of 14.4  $\pm$  0.08‰ ( $n = 3$ , in 1970) and 14.3‰ (in 1972) (Fig. 3). From 1987 to 1994,  $\delta^{15}\text{N}_{\text{Isaza}}$  varied from 16.7‰ to 15.2‰ and were almost constant or slightly decreasing.

Organic carbon and nitrogen contents of surface sediments were 51.0 and 5.83 mg g<sup>-1</sup> dry sediments (mg g<sup>-1</sup> ds), respectively. They rapidly decreased to 19.9 and 2.42 mg g<sup>-1</sup> ds at 3.0 cm depth (in 1952; Table 2). From 3.0 to 8.5 cm (1952 to 1901), the organic carbon and nitrogen contents gradually decrease from 19.9 to 13.3 mg g<sup>-1</sup> ds in carbon and from 2.42 to 1.75 mg g<sup>-1</sup> ds in nitrogen. The C:N molar ratio ranged from 11.0 to 8.9. The up-core nitrogen isotope record ( $\delta^{15}\text{N}_{\text{sediment}}$ ) showed a remarkable increase, from 3.8‰ in 1901 to 8.1‰ in 1995 (Fig. 3). In the lower portion of the core  $\delta^{15}\text{N}_{\text{sediment}}$  values increased 1.1‰ from 1901 to 1956 (0.02 ‰ yr<sup>-1</sup>). In contrast, the upper portion of the core (from 1956 to 1995) had an increased rate of  $\delta^{15}\text{N}_{\text{sediment}}$  change of 0.08 ‰ yr<sup>-1</sup>.

**Diagenetic alterations of sedimentary nitrogen isotope ratio**—Degradation of organic matter in the water column and sediments has the potential for producing alterations of  $\delta^{15}\text{N}$  values. As for this diagenetic alteration of  $\delta^{15}\text{N}$  signature, there has been opposing evidence so far. On the basis of the analytical results of suspended particulate matter from the Pacific Ocean, Saino and Hattori (1980) suggested that the breakdown of organic matter may preferentially release isotopically “light” nitrogen with subsequent enrichment of the remainder. Several later studies confirmed their results (e.g., Altabet 1988). On the other hand, sediment trap and sediment studies indicated that  $\delta^{15}\text{N}$  values of sinking particles remained unchanged in the water column and were virtually the same as  $\delta^{15}\text{N}$  of sediments when organic preservation is

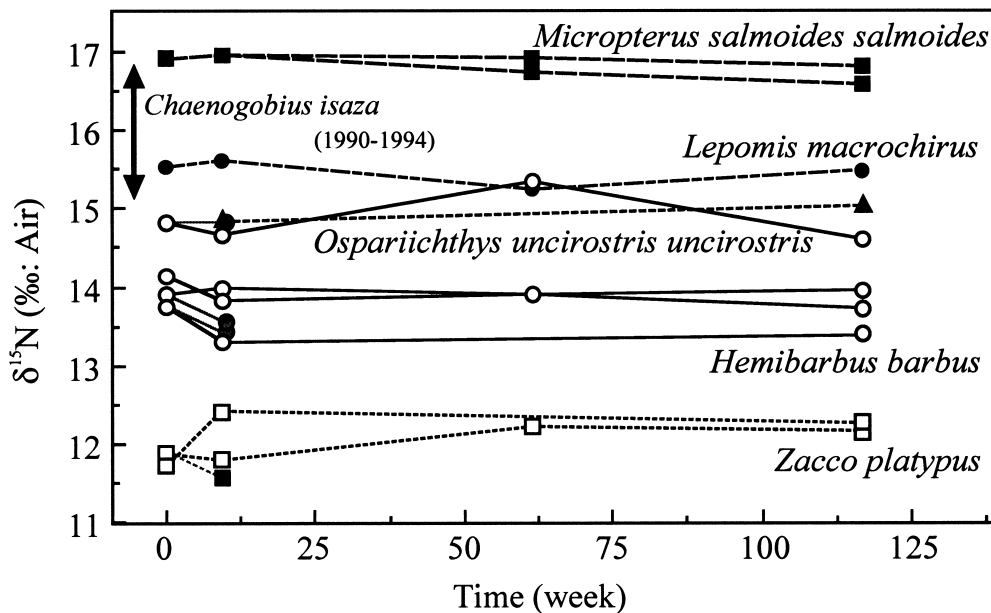


Fig. 2. Nitrogen isotope ratios of formalin fixed and unfixed fish specimens (*Micropterus salmoides*, *Lepomis macrochirus*, *Ospariichthys uncirostris uncirostris*, *Hemibarbus barbus*, and *Zacco platypus*) collected from Lake Biwa. The periods of formalin fixation of these specimens are 0 (unfixed), 9, 60, and 117 weeks. Shaded symbols indicate ethanol-fixed (9-weeks) samples. Double arrow indicates a range of nitrogen isotope ratios of Isaza fish specimens collected from 1990 to 1994.

moderate or better (Lake Michigan: Meyers and Eadie 1993; Lake Ontario: Hodell and Schelske 1998, eastern North Pacific: Altabet et al., 1999).

In Lake Biwa, the  $\delta^{15}\text{N}$  value of surface sediments (8.1‰) is nearly identical to the mean  $\delta^{15}\text{N}$  value of particulate organic matter (POM) samples that were collected from near the core site in the north basin during 1992–1995 (8.2‰, Yamada et al. 1998). This fact suggests that, at least in our core site, the  $\delta^{15}\text{N}$  value of surface sediments faithfully records the mean  $\delta^{15}\text{N}$  of organic matter produced in the surface water, whereas diagenetic alteration of  $\delta^{15}\text{N}$  values in the water column and on the lake floor is relatively small. Furthermore,  $\delta^{15}\text{N}_{\text{sediment}}$  record fits well with  $\delta^{15}\text{N}_{\text{Isaza}}$  (Fig. 3) with a mean difference of 8.6‰, in which the offset is due to differences in  $\delta^{15}\text{N}$  trophic level. Therefore, in the following discussion, we assume that the diagenetic alterations of  $\delta^{15}\text{N}_{\text{sediment}}$  is negligibly small throughout the sediment column.

*Factors controlling present Isaza's  $\delta^{15}\text{N}$  value*—As consumers assimilate and excrete nitrogen, isotope fractionation occurs in which  $\delta^{15}\text{N}$  increases with increasing trophic level (Minagawa and Wada 1984). The enrichment of  $^{15}\text{N}$  in animals of successive trophic levels has been reported in many marine and terrestrial ecosystems (e.g., Wada et al. 1987; Fry 1988; Cabana and Rasmussen 1994). According to Minagawa and Wada (1984), the mean enrichment factor of  $\delta^{15}\text{N}$  in the body tissue of animals is 3.4‰ per trophic step. Although it varies in some ecosystems, Yamada et al. (1998) reported that in Lake Biwa the  $\delta^{15}\text{N}$  enrichment factor in the north basin is 3.3‰ per trophic step, which is nearly iden-

tical to the value reported by Minagawa and Wada (1984). Therefore,  $\delta^{15}\text{N}$  of Isaza fish ( $\delta^{15}\text{N}_{\text{Isaza}}$ ) can be correlated with the nitrogen isotopic ratio of phytoplankton ( $\delta^{15}\text{N}_{\text{pp}}$ ) in the following equation:

$$\delta^{15}\text{N}_{\text{Isaza}} = 3.3 (TL_{\text{Isaza}} - 1) + \delta^{15}\text{N}_{\text{pp}}, \quad (1)$$

where  $TL_{\text{Isaza}}$  is the trophic level of Isaza fish in Lake Biwa. Taking into consideration that the  $\delta^{15}\text{N}$  values of surface sediments are almost the same as those of phytoplankton and sinking particles (Yamada et al. 1998), Eq. 1 can be rewritten as

$$\delta^{15}\text{N}_{\text{Isaza}} \approx 3.3 (TL_{\text{Isaza}} - 1) + \delta^{15}\text{N}_{\text{sediment}}. \quad (2)$$

When we applied observed  $\delta^{15}\text{N}$  values in Eq. 2, the trophic level of Isaza was calculated to be  $3.6 \pm 0.2$ , and it has not drastically changed during the past 50 yr. The calculated trophic level of 3.6 is consistent with observed trophic status on the basis of the contents of their gut, which showed that Isaza fed mainly on zooplankton (*D. galeata*, *D. brachyurum*, *M. leuckarti*, and *L. kindtii*) and gammarids (*A. annandalei*) (Nakanishi and Nagoshi 1984). Although Nakanishi and Nagoshi (1984) reported that the zooplankton contribution to Isaza's diet was decreased (from 53.9% to 13.0%), whereas the contribution from gammarids was increased (from 22.1% to 64.3%) after 1970, their trophic level may not have changed much, because the main diet of both zooplankton and gammarids consists of phytoplankton, detritus, and, partially, smaller zooplankton. The mean difference in  $\delta^{15}\text{N}$  (1.1‰; 0.3 of  $TL$ ) between zooplankton (11.4‰) and gammarids (10.3‰) also supports this consideration (Yamada et al. 1998).

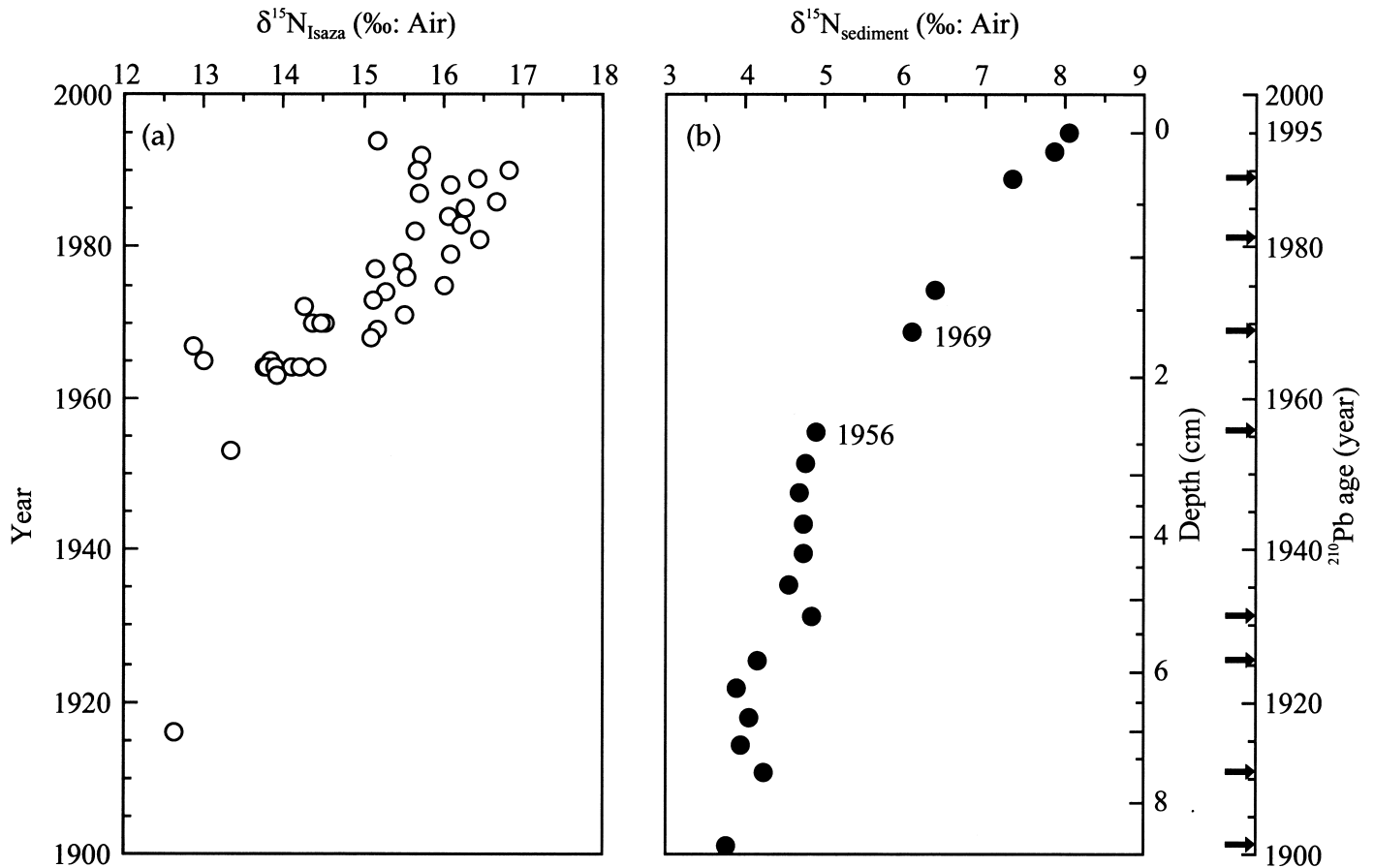


Fig. 3. Nitrogen isotope ratio of (a) Isaza fish specimens and (b) sediments collected from Lake Biwa. Isaza fish were collected in 1916, 1953, and every year from 1963 to 1994.  $\delta^{15}\text{N}_{\text{Isaza}}$  data are shown against their collected year.  $\delta^{15}\text{N}_{\text{sediment}}$  are shown against depth in core and  $^{210}\text{Pb}$  age. Arrows indicate the sections of  $^{210}\text{Pb}$  age determination (see text). Each symbol denotes the analytical result of an individual sample.

Laboratory and field observations have shown that phytoplankton preferentially uptake  $^{14}\text{N}$  over  $^{15}\text{N}$  (e.g. Wada and Hattori 1978; Montoya 1994). Thus, the  $\delta^{15}\text{N}$  value of phytoplankton ( $\delta^{15}\text{N}_{\text{PP}}$ ) can be correlated with that of dissolved inorganic nitrogen (DIN;  $\delta^{15}\text{N}_{\text{DIN}}$ ) and the amplitude of isotopic discrimination ( $\Delta$ ) at the time of assimilation. This correlation is shown in

$$\delta^{15}\text{N}_{\text{PP}} = \delta^{15}\text{N}_{\text{DIN}} + \Delta. \quad (3)$$

With the compilation of Eqs. 1 and 2 and a consideration of  $TL_{\text{Isaza}} = 3.6$ , we have

$$\delta^{15}\text{N}_{\text{Isaza}} = 8.6 + \delta^{15}\text{N}_{\text{DIN}} + \Delta. \quad (4)$$

Equation 4 indicates that the nitrogen isotopic ratio of Isaza fish can be estimated by two factors: the fractionation factor ( $\Delta$ ) and  $\delta^{15}\text{N}$  value of substrate DIN.

The isotopic discrimination by nitrogen assimilation by phytoplankton showed negative values ranging from 0 to  $-19\text{‰}$  (Montoya and McCarthy 1995; Waser et al. 1998). The degree to which isotopic discrimination occurs seems to depend on several factors such as the conditions under which samples were culture (Waser et al. 1998) and the type of algal species (Montoya and McCarthy 1995). Isotopic frac-

tionation in diatoms is larger ( $5.2\text{‰}$ – $12.1\text{‰}$ ) than that of flagellate species ( $0.9\text{‰}$ – $3.2\text{‰}$ ) (Montoya and McCarthy 1995; Waser et al. 1998). Dominant phytoplankton species in Lake Biwa were diatoms (*Aulacoseira solida* and *Stephanodiscus carconensis*) and green alga (*Pediastrum biwae*) until 1957 (Negoro 1981). They were replaced by other species of diatoms (*S. car. v. pusilla*) and green algae (*Clasterium* spp. and *Staurastrum* spp.) until 1965. A flagellate (*Uroglena americana*) took part in one of the dominant species after 1977 until the present (Negoro 1981). According to monthly observations of phytoplankton species, both green or blue-green algae and diatoms in Lake Biwa showed almost the same isotopic variations ( $4\text{‰}$ – $14\text{‰}$ ) in 1993–1994 (Yamada et al. 1998). Thus, we do not have plausible explanation for the dramatic increase in the degree to which isotopic fractionation occurred in the mid-1960s, even though there have been changes in the major planktonic communities during the latter half of the 20th century. In Lake Biwa, the dominant form of DIN is nitrate, whereas concentrations of nitrite and ammonium are negligibly small (Tezuka and Nakanishi 1991; Shiga Prefecture 1997). When the water column is stratified (from April to December), nitrate is almost exhausted in the epilimnion, whereas it is

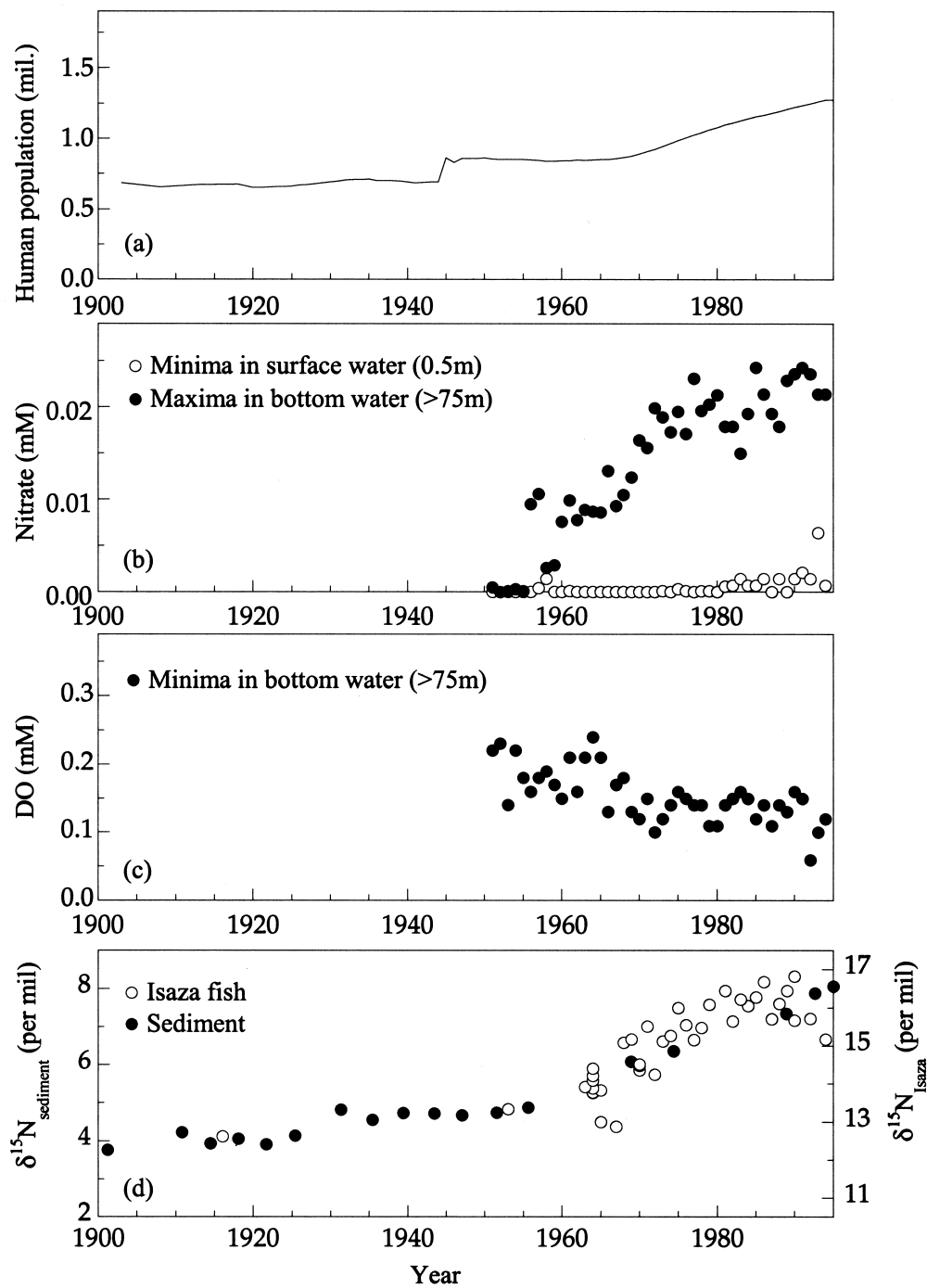


Fig. 4. Fluctuations in (a) human population of the watershed, (b) nitrate and (c) dissolved oxygen (DO) concentration, and (d) observed  $\delta^{15}\text{N}$  records of Lake Biwa. Samples for nitrate and DO were collected from surface (0.5 m) and bottom (>75 m) layers in the north basin of Lake Biwa during the past several decades. Nitrate concentrations are annual minima and maxima of monthly observational data in surface and bottom waters, respectively (Shiga Prefecture 1951–1997). DO concentrations are that of annual minima in bottom waters (Shiga Prefecture 1951–1997).

present in high concentrations (15–25  $\mu\text{M}$ , min.–max. from 1990 to 1995) in the hypolimnion. Because nitrate in epilimnion is mostly utilized on an annual basis (Fig. 4), there is no or little impact on the  $\delta^{15}\text{N}$  of sediment or fish. Therefore,

we conclude that the  $\Delta$  factor could not explain the 3‰ increase of  $\delta^{15}\text{N}_{\text{Isaza}}$  and  $\delta^{15}\text{N}_{\text{sediment}}$  after the mid-1960s.

From 1952 to 1994, the annual mean nitrate concentration in the bottom and surface waters of the north basin gradually

increased from 2.7 to 18.7  $\mu\text{M}$  and from 2.7 to 9.0  $\mu\text{M}$ , respectively (Fig. 4). These recent increases in nitrate are due to enhanced input of anthropogenic nitrogen to the lake, which is related to the population increase within the watershed (Fig. 4). The enhanced anthropogenic inputs from domestic sewage and fertilizer have the potential to increase the  $\delta^{15}\text{N}_{\text{DIN}}$  values of the lake. Unfortunately, we cannot currently critically verify this possibility, because we do not have a historical extent of enhanced anthropogenic inputs and we do not know the  $\delta^{15}\text{N}$  values of domestic sewage and fertilizer. Nitrogen fertilizer, such as ammonium sulfate, is chemically synthesized from atmospheric nitrogen and may have  $\delta^{15}\text{N}$  values close to the atmospheric nitrogen. Yamada et al. (1996) reported that a mean  $\delta^{15}\text{N}$  of nitrate at the mouth of Ane River, one of the largest rivers that flows into the north basin, was as low as 2.0‰ in 1993–1994. This suggests that nitrate in river water that contains discharge from sewage plants would not be a main cause for the  $\delta^{15}\text{N}$  increase in recent Lake Biwa.

However, it was reported that increased applications of nitrogen fertilizer led to an increase in soil denitrification and significantly increase  $^{15}\text{N}$  in groundwater runoff (Cabana et al. unpubl.), because denitrification preferentially removes  $^{14}\text{N}$  (e.g., Cline and Kaplan 1975; Wada and Hattori 1991). Recent groundwater flux to Lake Biwa was estimated at 0.16–1.32  $\text{km}^3 \text{yr}^{-1}$  (Somiya 2000), which corresponds to 0.6%–4.8% of the total water budget of Lake Biwa. Therefore, we consider that the increase of  $\delta^{15}\text{N}_{\text{DIN}}$  could be partially explained by an enhanced input of nitrogen fertilizer and increased soil denitrification though runoff, although we do not have any reliable historic  $\delta^{15}\text{N}$  data from the watershed of Lake Biwa.

An alternative explanation for the increase in  $\delta^{15}\text{N}_{\text{DIN}}$  is enhanced denitrification in Lake Biwa, which preferentially removes  $^{14}\text{N}$  from the lake. According to previous studies (reviewed by Yoshioka 1991), only 40%–47% of nitrogen supplied to the lake was flushed out by the Seta River during the 1980s. Furthermore, Miyajima (1994) estimated that denitrification removed up to 50% of the total nitrogen input to Lake Biwa on the basis of a year-round (1991–1992) observation of sedimentary nitrogen components and mass balance calculations. He suggested that the large contribution of denitrification in recent times is due to the reduction of availability of oxygen at the sediment/water interface when the lake is stratified. Yamada et al. (1996) confirmed this view on the basis of an isotope balance calculation focused on nitrate nitrogen. In general, denitrification occurs in oxygen-depleted environments in deep sediments and within microsites dispersed inside oxic surface sediments (e.g., Seitzinger 1988). Denitrification could be categorized into two types by their environments. The first denitrification occurs within the sedimentary anoxic layer, where the denitrification rate is limited by the diffusion of nitrate through the sediment. The second denitrification occurs in the hypolimnion without the limitation of nitrate. In Lake Biwa, high productivity in the epilimnion, accompanied with eutrophication, increased organic matter input and oxygen consumption in hypolimnion. In fact, dissolved  $\text{O}_2$  concentrations in the hypolimnetic water during the latter months of the stratified period (October–December) showed a 50% decline,

from 0.28 mM in 1964 to 0.13 mM in 1972 (Shiga Prefecture 1958–1997), which corresponds to the timing of  $\delta^{15}\text{N}_{\text{Isaza}}$  increase (Fig. 4). In such case, increased rates of denitrification in seasonally anoxic hypolimnion water can contribute to  $\delta^{15}\text{N}$  increase in the lake.

*Conclusions*—The remarkable increase in  $\delta^{15}\text{N}$  recorded in muscle tissue of Isaza fish and sediments since the mid-1960s is ascribed to an increase in  $\delta^{15}\text{N}$  of nitrate due to the enhanced denitrification process in groundwater or lake water. Other factors, including diagenetic alteration and fractionation factors during the assimilation of nitrate, had a minor effect on the  $\delta^{15}\text{N}$  values. In museums and universities, fish specimens have often been collected from many lakes and stored in ethanol after formalin fixation for a long period of time. We propose that  $\delta^{15}\text{N}$  of these fish specimens can be useful for tracing and understanding anthropogenically induced eutrophication.

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