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Food sources for the bivalve *Corbicula japonica* in the foremost fishing lakes estimated from stable isotope analysis

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ABSTRACT: Carbon and nitrogen stable isotope ratios in tissue of the bivalve corbicula *Corbicula japonica* and particulate organic matter (POM) were measured in Lake Jusan, Lake Ogawara and Lake Shinji, which are the foremost fishing grounds for the corbicula in Japan, to determine their food sources. The bivalves in Lake Ogawara and Lake Shinji showed enriched isotope composition, while those in Lake Jusan were depleted. In addition, the difference in the isotope ratios between the sampling sites was remarkable in Lake Jusan. Chlorophyll concentrations were significantly higher in Lake Ogawara and Lake Shinji than those in the inflow rivers, although that in Lake Jusan was equivalent to that in the river. Residence time of river water was estimated at 1 day, 455 days and 88 days in Lake Jusan, Lake Ogawara and Lake Shinji, respectively. These values indicate that the bivalves in Lake Ogawara and Lake Shinji assimilate autochthonous phytoplankton, while those in Lake Jusan assimilate terrestrial matter in the upper reaches and marine phytoplankton in the lower reaches because of low production in the lake.

KEY WORDS: **bivalve, brackish lake, *Corbicula japonica*, food source, particulate organic matter, stable isotope.**

INTRODUCTION

The infaunal suspension-feeding bivalve corbicula *Corbicula japonica* inhabits brackish waters. The landing of corbicula in 2001 reached 17 000 tons, which was 28% of the total landings of inland fisheries, so that the corbicula is one of the most important fishery resources in Japan. Corbicula plays an important role in the ecosystem through feeding and nutrient excretion activities, because they often dominate the macrobenthic community in brackish waters.[1,2] It is therefore essential to know corbicula nutrition for the elucidation of carbon and nitrogen cycling in the ecosystem. Estimates of food sources are also important for the management of the corbicula resources, because it would affect their growth rates and reproduction.

Corbicula obtains particles through filtration by holding its inhalant siphon above the sediment surface. There are several ways to understand the diet of organisms. However, among the methods, direct observation of feeding behavior of bivalves is unfeasible over long periods in the field. Indirect techniques, such as gut content analysis of bivalves may be misleading because it cannot distinguish ingested material that is not assimilated. Algae, for example, can survive passage through the digestive tract.[3] Therefore, neither direct observation nor gut content analysis are suitable for determining food sources of corbicula.

In contrast to these conventional methods, stable isotope analysis has received increasing interest as it can measure assimilation by potential producers. The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of an animal directly reflect the composition of food sources assimilated and incorporated over time.[4] Since the isotope composition in each primary source of organic matter shows different characteristics, this method has been used successfully in many studies of spatial and temporal variations in potential diets of invertebrates in various estuarine and saltmarsh food webs.[5] In our previous paper, carbon and nitrogen isotope ratios were measured in the body of *C. japonica* in the lower reaches of

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the Kushida River.[6] The results indicated that the contribution of terrestrial organic matter is significantly important for the corbicula diet, although the contribution gradually changes among sampling sites.

Corbicula japonica inhabits lower reaches of rivers and brackish lakes, so that the corbicula fisheries are prospering in many lakes. The environmental conditions in brackish lakes are usually different from those in river mouths. From the physical point of view, for example, it is expected that surface water temperature is higher in brackish lakes than river mouths in summer. However, active primary production caused by the long residence time of water might increase abundance of phytoplankton and lead to improved biological conditions in brackish lakes.[7] The relative contributions of food sources of the corbicula in the river mouth were quantitatively estimated by Kasai and Nakata,[6] but those in brackish lakes are still unknown. The differences in the environmental conditions would cause different contributions between rivers and lakes. Therefore, the present study investigates the isotopic composition of *C. japonica* in brackish lakes. Based on the isotopic results, the use of different components of organic matter in the bivalves' diet is discussed in relation to the difference in the environmental conditions among brackish lakes and lower reaches of rivers.

MATERIALS AND METHODS

This study was conducted in Lake Jusan, Lake Ogawara and Lake Shinji, which are the foremost fishing grounds for corbicula in Japan (Fig. 1a).

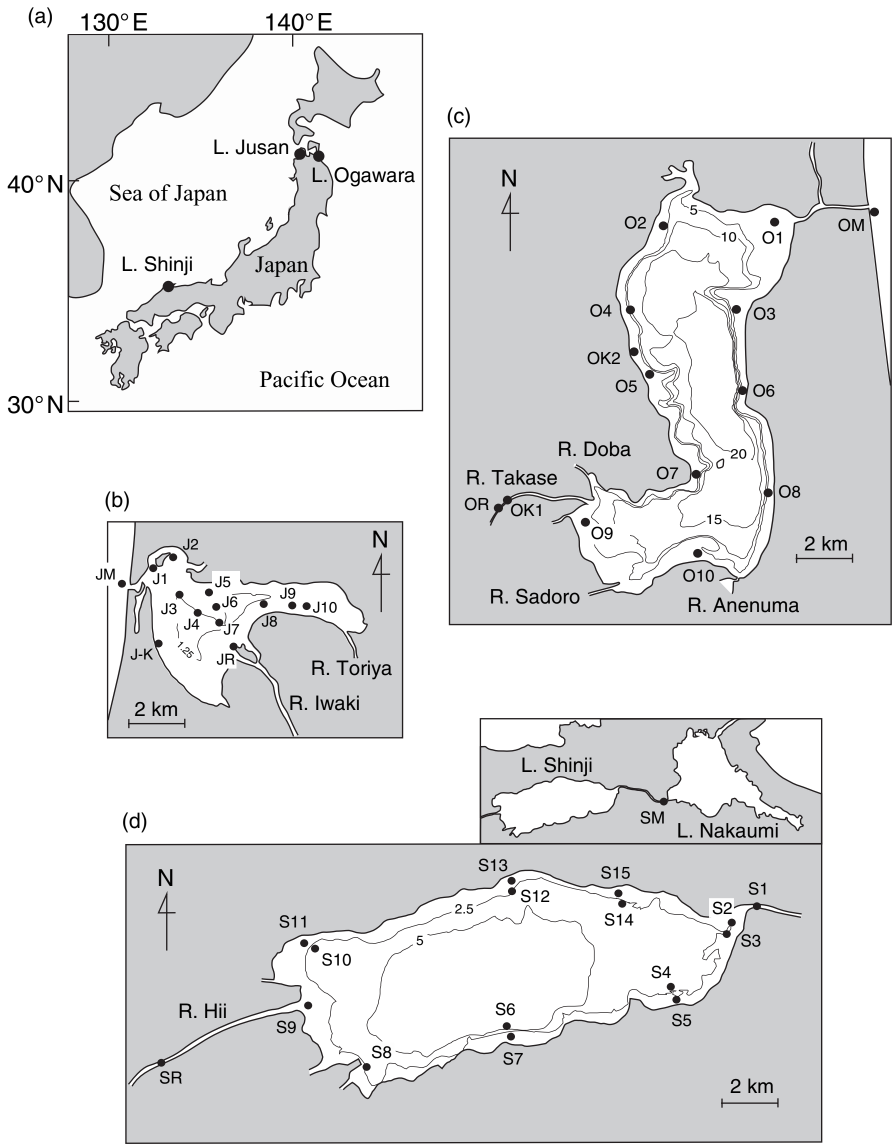

Fig. 1 (a) Site locations. Topography and sampling stations in (b) Lake Jusan, (c) Lake Ogawara and (d) Lake Shinji. K, M and R in the labels indicate stations from which benthic microalgae were collected, marine stations and riverine stations, respectively. Depth contours in lakes are in meters.

Landing in the three lakes accounted for over 70% of the total corbicula landing in Japan in 2001. The corbicula is the main component of the animal biomass in each lake, and thus the most important fisheries resources.

Lake Jusan is a shallow mesohaline lake situated on the coast of Sea of Japan (Fig. 1b). The lake has an area of 18.1 km^2, and an average depth of 2 m with a maximum of 3 m. The River Iwaki and River Toriya empty into the lake at the south-eastern and eastern ends, respectively. Adequate water exchange between the lake and ocean through the north-western channel leads higher salinity in the north-west (>5), while salinity is kept lower by the freshwater discharge in the southern and eastern area (<5).[8] Corbicula mainly inhabits the northern part of the lake.[9]

Lake Ogawara is a deep oligohaline lake on the coast of Pacific Ocean (Fig. 1c). It has an average depth of 11 m with a maximum of 25 m in the central part. The lake is approximately 4 km wide and 14 km long with a surface area of 65.6 $km.^2$ Fresh water is supplied mainly from the River Takase with additional supply from the River Sadoro, River Doba and River Anenuma at the southern or south-western ends, while the lake water flows out to the Pacific Ocean at the north-eastern end. The salinity in the lake is significantly low (~1) because water exchange between the lake and ocean is strongly limited.[9] Corbicula mostly inhabits shallow coastal area of the whole lake.

Lake Shinji is a shallow oligohaline lake on the coast of the Sea of Japan (Fig. 1d). The surface area of Lake Shinji is 79.2 km^2 and its mean depth is 5 m with a maximum depth of 6 m at its center. Freshwater mainly derives from the River Hii at its west end, with relatively small freshwater input from minor rivers around the lake. The lake water flows out to mesohaline Lake Nakaumi, which is connected to the Sea of Japan, through a narrow channel at the east end. Salinity in Lake Shinji is usually <10, while that in Lake Nakaumi is >15.[10] The corbicula does not inhabit the central part but inhabits the coastal area <4 m depth because of the hypoxia in the former in summer.[11]

Corbicula sampling locations are shown in Figure 1. Live bivalves were collected at 10 points in Lake Jusan on 20–21 July, at 10 points in Lake Ogawara on 16–18 July, and 15 points in Lake Shinji on 3–5 August 2004 (Fig. 1). A dredge net or joren, which is commonly used for corbicula fisheries in Japan, was used for bivalve collections in Lake Jusan and Lake Ogawara, while a Smith–McIntyre grab sampler (Rigo, Saitama, Japan) was used in Lake Shinji. Six corbicula were collected at each point in a total of 210 individuals. The shell lengths of corbicula were 25.4 ± 1.87 mm, 28.2 ± 2.00 mm and 23.7 ± 2.93 mm (average ± standard deviation) in Lake Jusan, Lake Ogawara and Lake Shinji, respectively, and their differences between stations in each lake were small. Bivalve samples were stored at –40°C until analysis. The foot muscle for each sample was excised, dried in an oven at 60°C, and ground to a fine powder with a mortar and pestle.

To investigate the characteristics of organic sources from rivers and lakes, the isotopes for particulate organic matter (POM) were also measured. For POM sampling in the lakes, 1 L of bottom water (0.5–1 m above the bottom) was collected with a van Dorn water sampler from stations where the bivalves were sampled. Fresh water at the middle or lower reaches of River Iwaki, River Takase and River Hii was also sampled (JR, OR and SR, Fig. 1) to provide data on terrestrial particulate organic matter that is transported to the lakes. In addition, as representatives of organic matter produced in the lower side of each lake, the water at the downstream side of the lakes was collected (JM, OM and SM, Fig. 1). However, these sampling sites may not offer pure marine POM, but potentially offer various mixtures of riverine, lake and marine POM. The POM was defined by the particles collected on a precombusted Whatman GF/F glass microfiber filter. In addition to terrestrial organic matter and phytoplankton, benthic microalgae have recently been recognized as important food sources for estuarine secondary producers by their high palatability.[6,12–14] Therefore, benthic microalgae were extracted from the surface sediments of the mudflat in Lake Jusan (JK) and Lake Ogawara (OK1 and OK2) by a procedure of Yokoyama and Ishihi.[12] The filter samples were put in a desiccator with HCl fumes for the first 24 h to eliminate $CaCO_3$, with NaOH fumes for the next 24 h to neutralize the acid, and then dried.

Stable isotope ratios of carbon and nitrogen were measured by a continuous-flow isotope-ratio mass spectrometer with an elemental analyzer (Carlo Erba, Lakewood, CO, USA) connected to a mass spectrometer (Finnigan MAT, Bremen, Germany). Isotope ratios, $\delta^{13}C$ and $\delta^{15}N$, are expressed by the standard δ notation (in ‰) in equation 1:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3 \quad (1)$$

where X is either ^{13}C or ^{15}N, and R is $^{13}C/^{12}C$ for carbon and $^{15}N/^{14}N$ for nitrogen. Pee Dee Belemnite and atmospheric nitrogen were used as the isotope standards for carbon and nitrogen, respectively. Precision in the overall preparation and analysis was ±0.12‰ for $\delta^{13}C$ and ±0.16‰ for $\delta^{15}N$.

An aliquot of water (0.2 L) was collected to measure the chlorophyll *a* (Chl-*a*) concentration in the lakes and rivers. Chl-*a* pigments were extracted from POM in the dark for 12 h by 90% acetone, and their concentrations were measured by solvent extraction photofluorometry.[15]

Salinity was observed at each sampling station using a water quality meter Clorotec (Alec Electronics, Kobe, Japan) in Lake Jusan and Lake Ogawara, and using a water quality sonde Quanta (Hydrolab, Loveland, CO, USA) in Lake Shinji. Since corbicula inhabits bottom sediments, only the bottom salinity was analyzed in this study.

The following data were also used to estimate the retention time of river water in each lake. Aomori Prefectural Fisheries Research Center observed salinity along the longitudinal lines of Lake Jusan and Lake Ogawara, off Lake Jusan (41°00′N, 140°00′E) and off Lake Ogawara (41°00′N, 141°30′E), once a month. In addition, Shimane Prefectural Inland Fisheries Experimental Station observed salinity by grid surveys in the whole area of Lake Shinji and Lake Nakaumi, once a month. The average salinity from April 1996 to November 2004 in Lake Jusan, from April 1996 to October 2004 in Lake Ogawara, from February 1995 to December 2004 off Lake Jusan, from June 1997 to November 2004 off Lake Ogawara, and from April 1995 to March 2002 in Lake Shinji and Lake Nakaumi were analyzed in this study. The discharge from the rivers flowing into each lake were quoted from the Japan Infrastructure Development Institute database.[16]

RESULTS

First, averages of stable isotope values for the corbicula were calculated at each sampling point. Second, they were classified by salinity into two or three groups in each lake; upper, middle and lower area. Third, their Mahalanobis distances from the center of each group were calculated. When the distance in the group was larger than that in another group, the bivalves were reclassified into the other group. The results are summarized in Table 1. Corbicula shows different isotope ratios in the different regions of the lakes, indicating different mixes of organic matter sources as their food. The δ^{13}C–δ^{15}N map of stable isotope composition clearly displays the characteristics (Fig. 2). In Lake Jusan, they were statistically classified into two groups. Nitrogen and carbon isotope ratios for the bivalves near inflow rivers (Stations J7, J9 and J10) were considerably depleted ($\delta^{13}C = -26.3 \pm 0.72$‰ and $\delta^{15}N = 9.0 \pm 0.66$‰), while those downstream (Stations J1–J6 and J8) were enriched in $\delta^{13}C = -22.7 \pm 0.59$‰ and $\delta^{15}N = 11.0 \pm 0.63$‰ (Fig. 2a, Student's *t*-test, $P < 0.01$). Figure 2b shows that the carbon isotope ratios for the bivalves were higher

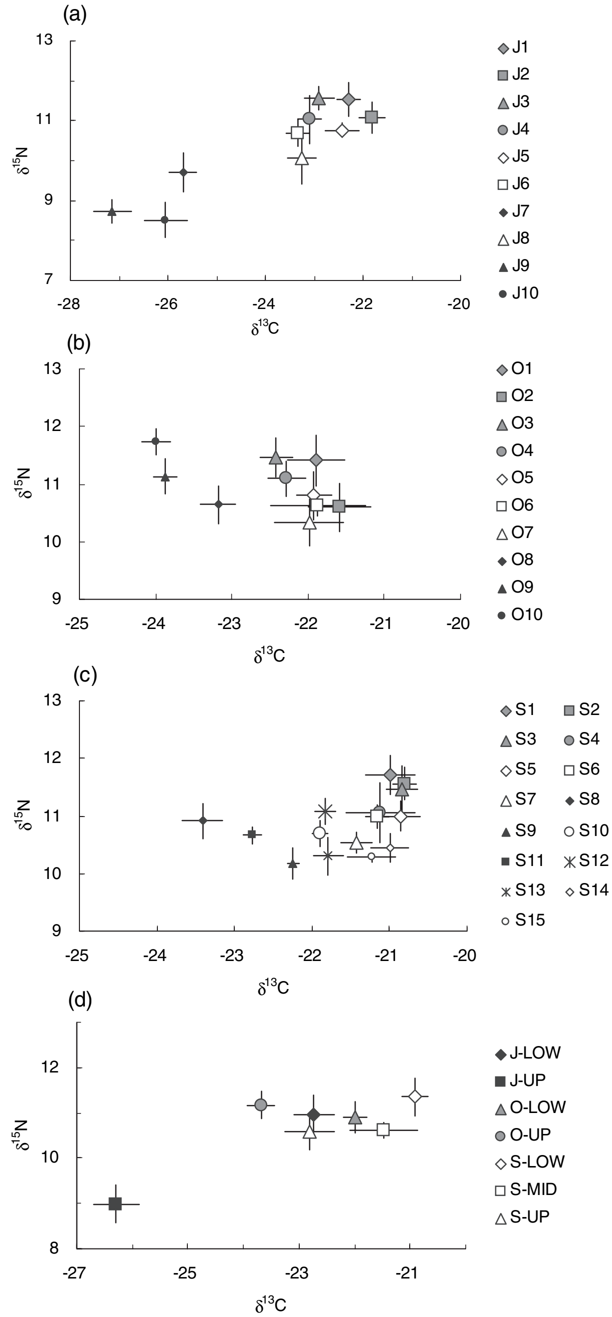

Fig. 2 δ^{13}C and δ^{15}N plot of *Corbicula japonica* in (a) Lake Jusan (b) Lake Ogawara (c) Lake Shinji and (d) all lakes. Bars indicate standard deviations. UP, MID and LOW indicate upper, middle and lower sites, respectively. See Table 1 for the definition of upper, middle and lower sites.

at Stations O8–O10 ($\delta^{13}C = -23.7 \pm 0.41$‰) than those at Stations O1–O7 ($\delta^{13}C = -22.0 \pm 0.44$‰) in Lake Ogawara. They were significantly different between upper and lower sites (Student's *t*-test, $P < 0.01$), although the variation was smaller than

Table 1 Carbon and nitrogen stable isotope ratios (‰) for the bivalves, salinity, chlorophyll concentrations and category of sampling points

	C. japonica				
Site	δ^{13}C (‰)	δ^{15}N (‰)	Salinity	Chl *a* conc. (μg/L)	Category
Lake Jusan					
J1	–22.3 ± 0.23	11.5 ± 0.43	7.17	7.3	Lower
J2	–21.8 ± 0.27	11.1 ± 0.39	6.63	4.6	Lower
J3	–22.9 ± 0.32	11.6 ± 0.29	nd	8.5	Lower
J4	–23.1 ± 0.25	11.0 ± 0.61	6.52	18.1	Lower
J5	–22.4 ± 0.35	10.8 ± 0.18	5.28	8.9	Lower
J6	–23.3 ± 0.24	10.7 ± 0.34	5.65	7.1	Lower
J7	–25.7 ± 0.28	9.7 ± 0.48	6.70	11.4	Upper
J8	–23.3 ± 0.29	10.1 ± 0.64	0.91	8.0	Lower
J9	–27.1 ± 0.38	8.7 ± 0.29	0.07	2.4	Upper
J10	–26.0 ± 0.44	8.5 ± 0.44	nd	5.1	Upper
Lake Ogawara					
O1	–21.9 ± 0.37	11.4 ± 0.44	1.48	27.5	Lower
O2	–21.6 ± 0.41	10.6 ± 0.42	1.39	30.6	Lower
O3	–22.4 ± 0.22	11.5 ± 0.33	1.30	23.9	Lower
O4	–22.3 ± 0.25	11.1 ± 0.30	1.39	24.7	Lower
O5	–21.9 ± 0.23	10.8 ± 0.41	1.39	26.9	Lower
O6	–21.9 ± 0.62	10.6 ± 0.18	1.29	37.4	Lower
O7	–22.0 ± 0.45	10.3 ± 0.40	1.34	27.2	Lower
O8	–23.2 ± 0.24	10.6 ± 0.33	nd	37.6	Upper
O9	–23.9 ± 0.15	11.1 ± 0.31	0.66	32.8	Upper
O10	–24.0 ± 0.18	11.7 ± 0.23	0.94	30.4	Upper
Lake Shinji					
S1	–21.0 ± 0.32	11.7 ± 0.34	6.46	8.2	Lower
S2	–20.8 ± 0.15	11.6 ± 0.29	6.32	17.0	Lower
S3	–20.8 ± 0.20	11.5 ± 0.42	6.53	17.5	Lower
S4	–21.1 ± 0.44	11.1 ± 0.52	6.16	14.3	Lower
S5	–20.9 ± 0.25	11.0 ± 0.26	5.91	22.6	Lower
S6	–21.2 ± 0.15	11.0 ± 0.19	5.09	20.8	Middle
S7	–21.4 ± 0.19	10.5 ± 0.18	3.89	28.1	Middle
S8	–23.4 ± 0.26	10.9 ± 0.30	4.23	46.1	Upper
S9	–22.2 ± 0.08	10.2 ± 0.27	3.21	19.2	Upper
S10	–21.9 ± 0.10	10.7 ± 0.23	5.93	14.2	Middle
S11	–22.8 ± 0.12	10.7 ± 0.15	5.79	7.8	Upper
S12	–21.8 ± 0.14	11.1 ± 0.23	6.11	13.3	Middle
S13	–21.8 ± 0.19	10.3 ± 0.33	6.05	9.9	Middle
S14	–21.0 ± 0.25	10.4 ± 0.18	6.53	14.0	Middle
S15	–21.2 ± 0.31	10.3 ± 0.08	6.53	11.1	Middle

Values in δ^{13}C and δ^{15}N columns are mean ± SD. Upper, Lower and Middle categories indicate Upper site, Lower site and Middle site, respectively, categorized by isotope values for the bivalves and salinity. nd, no data.

that in Lake Jusan. Nitrogen isotope ratios were 11.1 ± 0.54‰ in the upper and 10.9 ± 0.53‰ in the lower sites, and no significant difference was detected (Student's *t*-test, $P > 0.01$). The variation in the isotope ratios was modest in Lake Shinji (Fig. 2c). However, a similar carbon isotope trend, that exhibited an enrichment from the upper site to the lower site, to the other lakes was apparent, and the bivalves were classified into three groups; (i) δ^{13}C = –22.8 ± 0.52‰ and δ^{15}N = 10.6 ± 0.39‰ at Stations S8, S9 and S11; (ii) δ^{13}C = –21.5 ± 0.39‰ and δ^{15}N = 10.6 ± 0.36‰ at Stations S6, S7, S10, and S12–S15; and (iii) δ^{13}C = –20.9 ± 0.30‰ and δ^{15}N = 11.4 ± 0.46‰ at Stations S1–S5 (ANOVA, $P < 0.01$). In all lakes, the deviations at each station were small for both δ^{13}C and δ^{15}N.

Figure 3 shows isotopic compositions for POM and benthic microalgae. POM in Lake Jusan was low (δ^{13}C < –25‰ and δ^{15}N < 8‰), with more depletion in upper sites (Stations J6–J10). The isotope values were comparable to those for riverine POM (δ^{13}C = –30.3‰ and δ^{15}N = 3.3‰), indicating strong influence of terrestrial matter. However, benthic microalgae showed considerably enriched

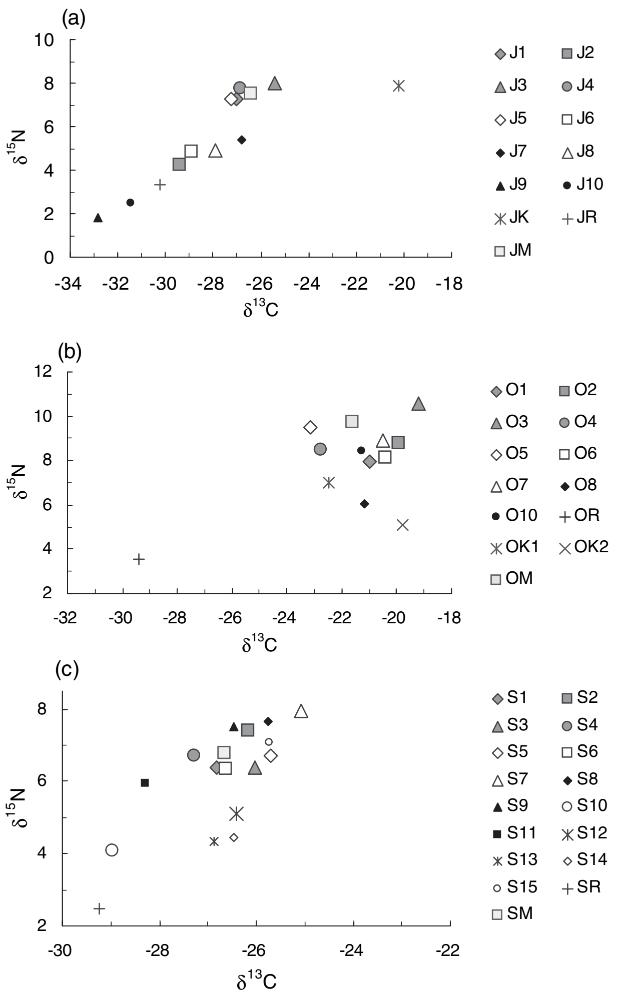

Fig. 3 δ^{13}C and δ^{15}N plot of particulate organic matter and benthic microalgae in (a) Lake Jusan (b) Lake Ogawara and (c) Lake Shinji. K, M and R in the labels indicate stations from which benthic microalgae were collected, marine stations and riverine stations, respectively.

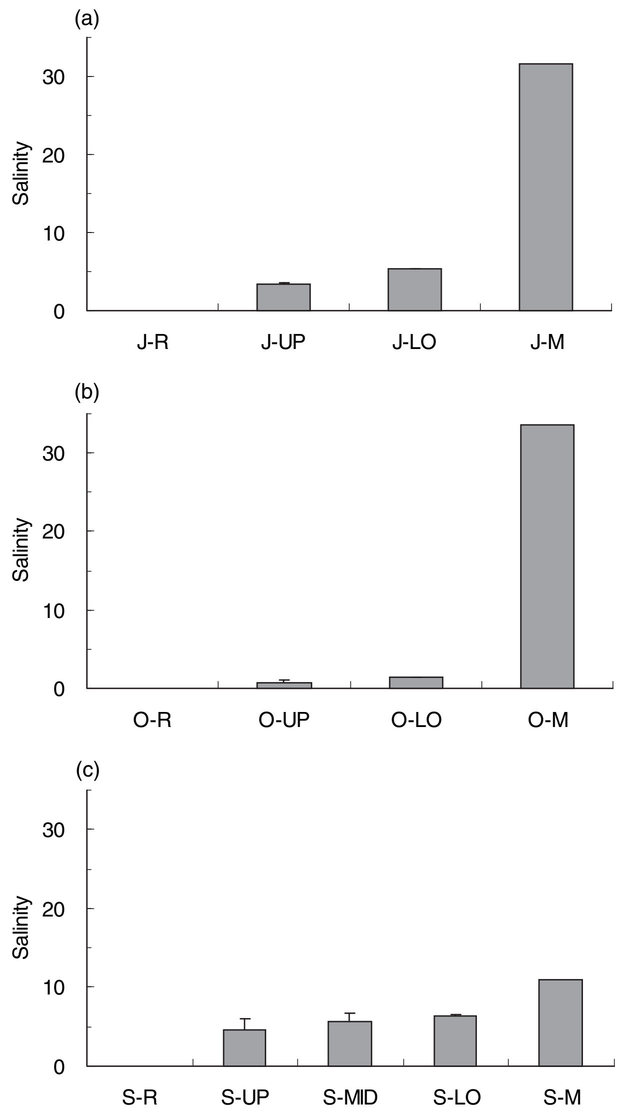

Fig. 4 Salinity in (a) Lake Jusan (b) Lake Ogawara and (c) Lake Shinji. UP, MID and LO indicate upper, middle and lower sites, respectively. Error bars, ±standard deviation.

ratios (δ^{13}C = –20.2‰ and δ^{15}N = 7.9‰). POM in Lake Ogawara had average δ^{13}C and δ^{15}N values of –21.0 ± 1.26‰ and 8.5 ± 1.23‰, respectively, with small variation. They are similar to the values for microalgae, and significantly enriched compared with POM in the inflow river. In Lake Shinji, isotope values at the western sites (Stations S10 and S11) were depleted (δ^{13}C < –28‰ and δ^{15}N < 6‰) and comparable to riverine POM values. POM at the center and the eastern sites showed higher values with small variation (δ^{13}C = –26.3 ± 0.60‰ and δ^{15}N = 6.5 ± 1.17‰).

Salinity was less than 1 at Stations J8 and J9, while 5.3–7.2 at Stations J1–J7 in Lake Jusan (Table 1 and Fig. 4a). The salinity front almost corresponded to the boundary between the upper and lower sites. The deviation in salinity was largest in the three lakes, similar to that for the isotope ratios for the bivalves and POM. In Lake Ogawara, salinity was low at all observation points, showing a trivial increasing trend from south to north (Fig. 4b). The western part of Lake Shinji showed slightly lower salinity (3.2–4.2 at S7–S9) than the central or eastern parts (5.1–6.5, Fig. 4c). It is a common feature in all lakes that salinity was lower in the upper sites than the lower, although the deviation was different among the lakes.

In Lake Jusan, Chl-*a* concentrations were 2.4–18.1 μg/L, which were comparable to that in the inflow river (5.8 μg/L, Table 1 and Fig. 5a), except

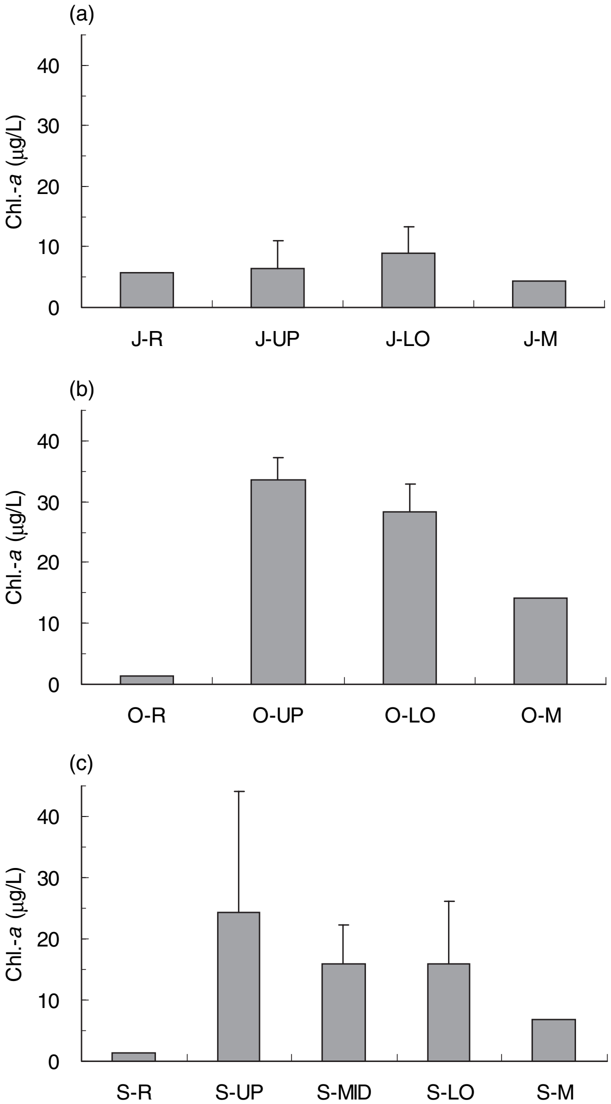

Fig. 5 Chlorophyll *a* concentrations in (a) Lake Jusan (b) Lake Ogawara and (c) Lake Shinji. UP, MID and LO indicate upper, middle and lower sites, respectively. Error bars, ±standard deviation.

for one point (J4) with a higher concentration. By contrast, Chl-*a* concentration was 1.3 μg/L in River Takase, but it rapidly increased in Lake Ogawara (23.9–37.6 μg/L, Fig. 5b). The trend in Chl-*a* concentration in Lake Shinji was similar to that in Lake Ogawara (Fig. 5c); Chl-*a* concentrations were considerably higher in the lake (7.8–46.1 μg/L) than that in the River Hii (1.3 μg/L). In addition, Chl-*a* concentration at the downstream sites of Lake Ogawara and Lake Shinji (Stations OM and SM) was lower than measurements inside the lakes. These results indicate that primary production is low in Lake Jusan, while remarkable in Lake Ogawara and Lake Shinji.

DISCUSSION

Salinity was >5 at Stations J1–J7, while <1 at Stations J8 and J9 in Lake Jusan (Table 1 and Fig. 4a). This result is consistent with previous reports,[8] and shows high salinity was restricted in the northwestern part of the lake. By contrast, the influence of fresh water was high in the southern and eastern parts of the lake. The water color changed between J6 and J8, showing a visible front between marine and fresh water. Each observation station in Lake Ogawara showed low salinity (<1.5, Table 1, Fig. 4b), indicating the least intrusion of ocean water into the lake. Using a numerical simulation and hydrographic observations, Ishikawa[17] showed that ocean water occasionally intrudes into the lake, but its influence is restricted to within a few kilometers from the channel and seldom spreads over the lake. Lower salinity was observed at Stations O9 and O10 than the other stations, indicating stronger influence of fresh water from the rivers. This is also consistent with the numerical simulation, which shows the river water is trapped by a circulation in the southern part of the lake.[17] Salinity exhibited a gradual increase from west to east in Lake Shinji (Table 1, Fig. 4c). The salinity distribution corresponds to flow patterns caused by the wind in summer.[18] Since the salinity gradient in Lake Shinji was modest compared with that in Lake Jusan, the fresh water from the river and saltier water from Lake Nakaumi would be efficiently mixed, although the circulation may affect the behavior of nutrient-rich fresh water.

It is generally supposed that primary production is low in Japanese rivers because the catchment basins are mountainous and thus the river water flows out speedily to the sea.[7] It is expected that phytoplankton would be abundant in lakes, which trap water for a long period. Assuming that water completely mixes in the lake, the retention time of river water (τ) is expressed by:

$$\tau = \frac{V(S_O - S_B)}{S_O R}, \qquad (2)$$

where V is the lake volume, S_O and S_B are the salinity outside and inside the lake, respectively, and R is the river discharge volume.[19] In this study, salinity in the outer ocean was used for S_O for Lake Jusan and Lake Ogawara, and that in the Lake Nakaumi for Lake Shinji. Substituting the values listed in Table 2 into equation 2, τ is estimated at 1, 455 and 88 days in Lake Jusan, Lake Ogawara and Lake Shinji, respectively. The residence time is considerably short in Lake Jusan, but those in the other two lakes are sufficiently long to grow phytoplankton. These estimates are in agreement with the observed chlorophyll concentrations in the lakes;

Table 2 Estimates of water retention time and parameters

Lake	V (m^3)	S_B	S_O	R (m^3/s)	τ (days)
Jusan	$1.8 \times 10^{7\dagger}$	17.3	33.6	88.2	1
Ogawara	$7.2 \times 10^{8\dagger}$	1.1	33.5	17.7	455
Shinji	$3.7 \times 10^{8\ddagger}$	4.4	21.0	38.4	88

V, volume of the lakes; S_B, salinity of the lakes; S_O, salinity outside the lakes; R, river discharge into the lakes; τ, retention time of river water. R for Lake Jusan is the river discharge of River Iwaki, for Lake Ogawara is that of River Takase, and for Lake Shinji is that of River Hii.

Data source †: http://www.thr.mlit.go.jp/ and ‡: http://www2.pref.shimane.jp/naisuisi/

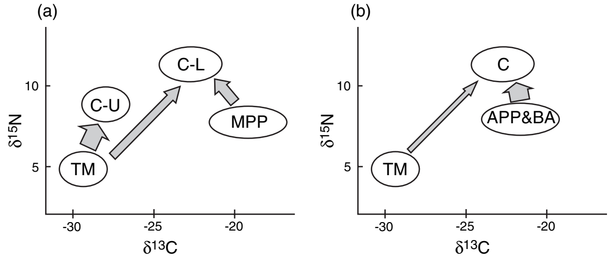

Fig. 6 Schematic view of isotope ratios and food webs in (a) Lake Jusan and (b) Lake Ogawara and Lake Shinji. C-U, corbicula in the upper sites; C-L, corbicula in the lower sites; C, corbicula; TM, terrestrial matter; MPP, marine phytoplankton; APP, autochthonous phytoplankton; BA, benthic microalgae. Wider arrows indicate larger flux of organic matter.

lower in Lake Jusan and higher in the others (Fig. 5). In a previous report,[20] the total nitrogen output from Lake Ogawara was estimated at 739 t/yr, compared with the total nitrogen input of 1303 t/yr. It is also accounted in Lake Shinji that the nitrogen output is about 70% of the input.[21] These results are consistent with our results on phytoplankton abundance. It is considered that phytoplankton grows actively using a plenty of nutrients flowing into the lakes from the rivers and is subsequently used by the secondary producers such as bivalves and zooplankton. However, residence time would be too short for growth of phytoplankton in Lake Jusan. In addition, the inflow river water contains abundant mineral particulates, so that high turbidity would restrict primary production in the lake.[8]

The significantly depleted isotope ratios for POM in the rivers are convenient for estimates of bivalve diets. The observed low values are in the range of the terrestrial organic matter,[22–24] indicating that riverine POM consists mainly of terrigenous C3 plants in the upper catchment basin. The loading of terrestrial matter has an effect on the isotope ratios for POM in Lake Jusan, which showed the most depleted values in the three lakes (Fig. 3a). The enriched carbon isotope ratios for benthic microalgae are also comparable with previous studies.[12,25–29] POM in Lake Ogawara showed higher δ^{13}C and δ^{15}N values than the other two lakes (Fig. 3b). Considering with the high productivity in the lake, the isotope ratios would reflect the autochthonous phytoplankton. However, determination of the relative contribution from phytoplankton and benthic microalgae to POM is difficult as the isotopic signatures are similar to each other. POM in Lake Shinji, in contrast to Lake Ogawara, showed a shift in the isotope ratios to that of terrestrial matter (Fig. 3c). This difference could result from heavy rain on the day before observation. Compared with a monthly average of approximately 5 mm/d in August, 74 mm/day precipitation was recorded on 2 August 2004 at Matsue on the east coast of Lake Shinji. A large amount of terrestrial matter must have flowed into the lake from the River Hii and other rivers. Therefore, the isotope ratios for POM changed temporally and the observed values would be unrepresentative of the normal POM in the lake. Previous measurement of isotope values, which showed more enriched δ^{13}C for sediments in Lake Shinji,[30] supports this hypothesis.

Both δ^{13}C and δ^{15}N values for corbicula were significantly depleted in the upper sites, while enriched in the lower sites in Lake Jusan (Fig. 2). The former was similar to the terrestrial POM. Since primary production is low in the lake, the contribution of terrestrial matter would be important as a food source for the corbicula in the upper sites (Fig. 6a). A C:N ratio of POM in the upper sites (average 11.8) which is higher than the Redfield ratio (~6.6) supports this idea. It is reported that δ^{13}C of marine phytoplankton is between –16 and –21‰, and δ^{15}N is between 7 and 10‰.[23,31,32] As δ^{13}C and δ^{15}N of animal tissue are ~1‰ and ~3‰ heavier than the diet, respectively,[4,33] the corbicula at the lower sites had stable isotope ratios between those for terrestrial matter and marine phytoplankton. Therefore, corbicula would assimilate both of them (Fig. 6a). The comparable isotope ratios of benthic microalgae and corbicula in the lower sites imply that microalgae might be one of the food sources. The contribution of benthic microalgae, however, should be insignificant in Lake Jusan because primary production is small. The dependence of terrestrial matter in the upper sites in Lake Jusan is similar to that in the lower reach of the Kushida River, where primary production would be small.[6] However, the corbicula in

Lake Ogawara and Lake Shinji had relatively enriched isotope values (Fig. 2d). Considering the isotope fractionation of 1‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$,[4,33] they are comparable to the isotope ratios for phytoplankton and/or benthic microalgae produced in the lakes (Figs 2,3). This means they would be the main food source for the corbicula, while the terrestrial matter would play a minor role in their nutrition (Fig. 6b). This food preference must be related to the high production in the lakes as was shown by the salinity distributions and high concentrations of Chl-*a*. However, slightly depleted $\delta^{13}C$ for the bivalves in the upper sites in each lake (Fig. 2) indicates a weak effect of the terrestrial matter on nutrition.

The wide variety of food sources for the corbicula presented in this and previous studies[6] might be peculiar to some animals inhabiting brackish waters. Many isotope studies have, by contrast, shown that the ecosystem in estuaries and coastal areas mainly depends on the marine produced phytoplankton and/or benthic microalgae, while riverine particulate organic matter appears to be a small component of food webs.[34–37] For example, *Ruditapes philippinarum* and *Mactra veneriformis*, both of which are common infauna in coastal areas, select marine POM from the organic matter available in their habitat, although most of the POM originates from terrestrial organic material.[31] It is explained that the terrestrial matter mainly consists of lignin and cellulose, which show refractory nature for biochemical degradation in coastal areas.[38] Therefore, most animals would either not select a large portion of terrestrial POM as it is unsuitable food matter, or the terrestrial matter remains indigested. However, depleted isotope signatures for corbicula indicate that it mainly uses lignin and cellulose as carbon sources. Recently, cellulase genes have been cloned from crayfish, mussel and abalone.[39–41] Future studies on the nutrition of various animals in brackish waters by isotope analysis, and consequent exploration of the cellulase gene from those species, including the corbicula, are suggested.

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