Review

Stable $\delta^{15}N$ and $\delta^{13}C$ isotope ratios in aquatic ecosystems

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Abstract: In the past 20 years, rapid progress in stable isotope (SI) studies has allowed scientists to observe natural ecosystems from entirely new perspectives. This report addresses the fundamental concepts underlying the use of the SI ratio. The unique characteristics of the SI ratio make it an interdisciplinary parameter that acts as a chemical fingerprint of biogenic substances and provides a key to the world of isotopomers. Variations in SI ratios of biogenic substances depend on the isotopic compositions of reactants, the pathways and kinetic modes of reaction dynamics, and the physicochemical conditions. In fact, every biogenic material has its own isotopic composition, its “dynamic SI fingerprint”, which is governed by its function and position in the material flow. For example, the relative SI ratio in biota is determined by dietary lifestyle, e.g., the modes of drinking, eating, and excreting, and appears highly regular due to the physicochemical differences of isotopomers. Our primary goal here is to elucidate the general principals of isotope partitioning in major biophilic elements in molecules, biogenic materials, and ecosystems (Wada, E. et al., 1995). To this end, the nitrogen and carbon SI distribution ratios ($\delta^{15}N$ and $\delta^{13}C$, respectively) are used to examine materials cycling, food web structures, and their variability in various kinds of watershed-including aquatic ecosystems to elucidate an “isotopically ordered world”.

Keywords: Natural C, N stable isotope ratios, fundamental concept of stable isotope method, application to aquatic ecosystems, food web

1. Introduction

General aspects of stable isotope techniques. Biogenic substances in nature contain significant amounts of stable isotopes (SI) of light elements such as hydrogen, carbon, nitrogen, and oxygen. For instance, a human body weighing 50 kg contains 225 g of heavier isotopes of light elements (Fig. 1).$^1$ Although the SIs of a particular element have rather similar chemical behaviors, their specific thermodynamic parameters and rate constants in chemical and biological reactions differ.

The SIs of biogenic substances vary depending on the isotopic compositions of the reactants, the pathways and kinetic modes of the reaction dynamics, and the physical and chemical conditions.

Thus, every biogenic material has its own unique isotopic composition, known as the “dynamic stable isotope fingerprint”,$^2$ which is determined by its function and position in the material flow of an ecosystem. In fact, the relative SI abundances in biota are determined by the common dietary lifestyle, e.g., the modes of drinking, eating, and excreting. The natural environment is, so to speak, the site of large-scale tracer experiments in time and space. Fundamental data as to the history of a molecule or a material can be obtained by precise determination of the isotopic ratios of the organisms in question.

Studies of this type should provide a new paradigm for understanding microscopic processes and macroscopic biological and biogeochemical phenomena in natural systems. In this manner, current biogeochemical and ecological problems can be analyzed, but only through highly accurate measurement of the relative SI abundance for any kind of ecosystem.

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A new paradigm. As described above, an ecosystem can be treated as the summation of complex chemical reactions mediated by functional living organisms and by physicochemical factors. In this type of system, the importance of the SI technique can be understood by taking an elemental reaction into consideration. For example, we start with the following simple chemical reaction:

\[ A \leftrightarrow A^* \rightarrow B \]  

[1]

In a closed system, the principal variables of this reaction are the change in molecular formula from molecule A to molecule B, the production or consumption of energy (\( \Delta G \)), and the difference in SI ratios between A and B resulting from isotopic effects on the reaction. The system pressure can also change due to variation in the partial molar volume of the chemicals in question. It can thus be emphasized that, like temperature and pressure, the isotope effect is a fundamental parameter that accompanies any chemical reaction; it allows a comprehensive understanding of the rate-limiting steps in reaction sequences involving unicellular algal physiology, the function and position of animals in an ecosystem food web, and their biogeochemical material cycles. The isotope effect parameter can be used at various levels from molecules to ecosystems and can be regarded as an interdisciplinary parameter of natural ecosystems.

Recent progress in life science has clarified that living organisms hold at least three chemical fingerprints within their body (Fig. 2). The first fingerprint is the genetic information obtained during the evolution of life over the past 3.5 billion years. The generally accepted “central dogma” of DNA says that genetic information is transferred from parents to offspring via a DNA fingerprint. DNA sequencing is an important technique in modern ecological studies, including studies of the evolution of life. The second fingerprint is the memory stored in animal brains. Elasticity of synaptic transmission is now considered to be a possible key to the mechanism of memory in the brain. Every animal accumulates different memories as it grows under different circumstances. However, this field lags in its understanding of the details.

The third fingerprint is the SI fingerprint, which is the most important parameter in SI studies. Biosynthesis can be described by the simple general reaction:

\[ \text{DNA} \rightarrow \text{RNA} \rightarrow \text{Protein} \]

\[ \uparrow \]

Substrate

Here, the isotopic compositions of the various substrates differ because of differences in food sources. The biosynthetic dynamics also affect the SI distribution among biopolymers. Consequently, each individual has its own isotope pattern or “SI fingerprint”. Heterogeneity in the intramolecular distribution of carbon isotopes has been successfully determined for malic acid (Fig. 3).

To sum up, all animal species have three chemical fingerprints, and differences in the SI ratios of biophilic elements in living organisms are a well-known phenomenon. The general aspects of SI techniques are thus emphasized:

1) SI studies give us a key to enter the new world of isotopomers;
2) SI ratios are interdisciplinary parameters relevant from the molecular level to the ecosystem level;
3) The SI ratio is one of the three chemical fingerprints of living organisms; and
4) SI ratio measurements can be made with high sensitivity (± 0.01%).

The SI differences between C3 and C4 plants and between land plants and marine phytoplankton in temperate regions are large enough that the \( \delta^{13} \)C
A value of an animal can be used as an indicator of its food web carbon source and that its $\delta^{15}N$ can be used to estimate its trophic level.\textsuperscript{4,5} Evidence is now accumulating to support the view that the biophilic elements in our biosphere occur in well-ordered distributions. This vision of the biosphere is known as the “isotopically ordered world”.\textsuperscript{2} In future SI studies, our primary goal is to elucidate the general principles of this world to provide a new paradigm for understanding our biosphere.

2. Materials and methods

Various biological and biogenic substances were collected from several aquatic ecosystems. Their N and C isotope ratios were measured using an isootope ratio mass spectrometer, as described elsewhere in detail.\textsuperscript{6,7}

$\delta$-notation. Since carbon and nitrogen isotope ratios vary only slightly in natural ecosystems, the $^{13}C$ and $^{15}N$ contents are expressed as per mil deviation from the standard, as defined below:

$$\delta^{13}C(\%e) = \left[ \frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{\text{PDB}}} - 1 \right] \times 1000 \quad [3]$$

$$\delta^{15}N(\%e) = \left[ \frac{(^{15}N/^{14}N)_{\text{sample}}}{(^{15}N/^{14}N)_{\text{air}}} - 1 \right] \times 1000 \quad [4]$$

Peedee belemnite, a fossil calcium carbonate that has a $^{13}C$ content nearly identical to that of HCO$_3^-$ in the ocean, and atmospheric nitrogen gas (N$_2$) are used as the standards. When a sample contains a higher $^{13}C$ or $^{15}N$ content than that of the corresponding standard, the $\delta$ per mil exhibits a positive value, the converse case yields a negative value. Here, $\delta^{13}C$ (HCO$_3^-$) = 0.0%e and $\delta^{15}N$ (N$_2$) = 0.0%e.

3. General considerations

The isotopically ordered world. Recent progress in this scientific field has clarified some general rules for partitioning of carbon and nitrogen isotopes in our biosphere. Isotope fractionation can be divided into two categories. The first category is isotope exchange equilibrium reactions. The most representative exchange reaction, by which the carbon isotope ratio of CO$_2$ in the atmosphere is kept almost constant all over the planet, is:

$$^{13}CO_2(\text{air}) + H^{12}CO_3^-(\text{sea}) \leftrightarrow ^{12}CO_2(\text{air}) + H^{13}CO_3^- \quad [5]$$

The second category is the kinetic isotope effect, which can take place ubiquitously in almost all biological reactions, depending upon the reaction mechanism. In general, the light isotopic
molecule in the molecular weight base has a larger rate constant in biochemical reactions than that of the heavier isotopic molecule. The fractionation magnitude depends on the inherent characteristics of the enzyme in question. For example, denitrification is well recognized to have a large nitrogen isotope fractionation factor of up to 1.04 (4%), whereas biological N\textsubscript{2} fixation has a small fractionation factor of 1.002 (0.2%). However, the fractionation factor of the same reaction in two different species is generally of the same magnitude if the enzyme active sites are similar. Consequently, the $^{15}\text{N}/^{14}\text{N}$ ratio of the biosphere as a whole is slightly higher than that of atmospheric nitrogen.\(^1\) On the other hand, a large isotope fractionation factor of 1.03 (3%) is observed for carboxylation in C3-plants, resulting in a lower $^{13}\text{C}/^{12}\text{C}$ ratio in the biosphere as a whole as compared with that of HCO\textsubscript{3} in the ocean.

The principal factors governing isotopic distributions in an ecosystem are the: (1) isotopic compositions of plant substrates such as CO\textsubscript{2}, H\textsubscript{2}O, and inorganic nitrogenous substances, (2) kinetic isotope effects during plant uptake processes; (3) branch reaction; (4) trophic effects during feeding processes; and (5) gas metabolism during mineralization (Fig. 4). In sum, plants exhibit regional characteristics in their isotopic compositions according to their circumstances. The carbon and nitrogen isotope ratios of an animal reflect its diet. The $^{13}\text{C}/^{12}\text{C}$ ratio of the animal is very close to that in its diet, whereas $^{15}\text{N}$ enrichment is well known among a variety of invertebrate and vertebrate animals. A correlation between the $^{15}\text{N}/^{14}\text{N}$ ratio of an animal and its corresponding trophic level has been clearly demonstrated in several ecosystems.\(^8,10\) Generally, C–O and N–O bond-cleavage reactions exhibit large isotope effects that provide a powerful tool for elucidating the origin or production pathway of CH\textsubscript{4} or N\textsubscript{2}O by their isotopic signatures.

Major empirical laws in nitrogen and carbon isotope ratios are summarized as follows:

1) \textit{Plants}: $\delta^{13}\text{C}$ is determined by the dynamics of CO\textsubscript{2} fixation during photosynthesis. C3 and C4 plants exhibit different $^{13}\text{C}$ contents.

2) \textit{Food chain}: The flow of organics along the food chain from the primary producers (food base) to animals at higher trophic levels has been reconstructed using both $\delta^{15}\text{N}$\(^3,8\) and $\delta^{13}\text{C}$\(^4,9\) values. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of animals during a single feeding process, the so-called “trophic effect”, is generally described by the equations:

![Fig. 4. Schematic model for isotopic ecosystem shown with emphasis on carbon and nitrogen isotope fractionation. The principal factors that govern the isotopic compositions of living organisms are: (i) the substrate isotope composition and kinetic isotope effects on uptake processes for plant (\(\mathbb{D}\)), (ii) the biochemical reactions occurring at branch point in the metabolic map (\(\mathbb{E}\)), (iii) the trophic effects during feeding processes (\(\mathbb{F}\)), and (iv) gas metabolism during mineralization (\(\mathbb{G}\)).](image)
Fig. 5. Schematic illustration showing the food web structures in Lake Baikal from isotopic viewpoints. The $\delta^{15}$N and $\delta^{13}$C levels of animals showed a clear trend of stepwise enrichment at each trophic level according to the following equations:

\[
\delta^{13}C(\text{animal}) \equiv (1.0 \sim 2.0)(\text{TL}-1) + \delta^{13}C(\text{algae}); \quad \text{and}
\]

\[
\delta^{15}N(\text{animal}) \equiv 3.3(\text{TL}-1) + \delta^{15}N(\text{algae}),
\]

where TL denotes trophic level (algal TL = 1).

3) An increase in $^{15}$N in an ecosystem is caused by evaporation of NH$_3$ and denitrification (NO$_3^-$ → N$_2$).

4. Results and discussion

Case studies in Lake Baikal. The distribution and variability of carbon and nitrogen isotope ratios ($\delta^{13}$C and $\delta^{15}$N) were investigated in food web structures in Lake Baikal. Recently, focus has been on the following subjects in order to elucidate the isotopically ordered world of Lake Baikal.

a) SI analyses of the pelagic food web: SI ratios of various organisms were analyzed to elucidate the food web structure. The pelagic food web structure is simple and consists of five major ecological groups: phytoplankton (Aulacoseira baicalensis), mesozooplankton (Epischura baicalensis), macrozooplankton amphipod (Macrohectopus branickii), fish (Coregonus autumnalis migratorius and four cottoid species), and seal (Phoca sibirica). Because of the low diversity and the consequently limited dietary options for each species, we were able to quantitatively estimate the dietary composition of each animal. Our carbon isotope data revealed that the $\delta^{13}$C levels in animals were similar to those in the pelagic phytoplankton, indicating that phytoplankton are the primary carbon source of the pelagic food web. The $\delta^{15}$N and $\delta^{13}$C levels of animals showed a clear trend of stepwise enrichment at each trophic level according to the following equations:

\[
\begin{align*}
\delta^{13}C (\%e) &= 1.8(\text{TL}-1) - 29.4\%e & [9] \\
\delta^{15}N (\%e) &= 3.3(\text{TL}-1) + 3.8\%e & [10]
\end{align*}
\]

We demonstrate that carbon and nitrogen SIs can be successfully applied to elucidate trophic relationships and conclude that the pelagic food web of Lake Baikal has an ideal, isotopically ordered structure (Fig. 5).

b) Baikalian omul [Coregonus autumnalis migratorius (Georgi)]: This fish has been caught in

Footnote:
— In ecology, the trophic level (TL) is the position that an organism occupies in a food web—what it eats and what eats it. By setting the TL of autotrophic organisms or “primary producers” (e.g., plant or algae) to be 1.0, TL of a given group of heterotrophic organisms or “consumers” is estimated from:

\[
\text{TL} = 1 + \text{mean TL of the food items},
\]

where the mean is weighted by the contribution of the different food items. Thus, for example, herbivorous species have TL = 2.0 while that of pure carnivorous species is larger than 3.0. TL of top predators in aquatic food webs often exceeds 6.
The deep-water benthic omul lives 350 m below the surface and feeds predominantly on pelagic amphipoda. Analysis of scales from 12-year-old omul showed a decrease in δ13C over time, reflecting changes in the δ13C of the atmospheric carbon dioxide, the omul’s ultimate source of body carbon. A periodic cycling of δ13C and δ15N in the scales during the last 50 years was detected. Sclerponge skeleton has been reported to faithfully record the recent trend of decreasing isotopic composition of atmospheric CO2. The omul δ13C results suggest that the recent shift of atmospheric δ13C not only affects autotrophic organisms but also permeates the entire ecosystem of pristine Lake Baikal (Fig. 6).

Our results, together with those from other collaborative studies with staff members of the Limnological Institute, SD-RAS, demonstrate that Lake Baikal provides an ideal, isotopically ordered laboratory for examining the effects of various circumstances on its ecosystems.

Case studies in a human dominated ecosystem: the Lake Biwa watershed. The Yodo River watershed is located in the central part of Honshu Island, Japan. This watershed is ca. 150 km in length, and its upper reaches include ca. 460 streams. Lake Biwa, which is located in the middle reach, is 65 km in length and holds 65% of the watershed’s water. The Yodo River begins in the lower reach and flows into Osaka Bay. The population density near the Yodo River is rather high, and extensive eutrophication is progressing toward the river mouth. Lake Biwa plays important roles in the biogeochemistry, ecology and sociology of the watershed. The northern basin is the major part of the lake, with a maximum depth of 104 m. The turnover time of the lake water is ca. 14.5 years. The basin is monomictic and mesotrophic. Lake Biwa has undergone large changes in nitrate concentration during the 20th Century as a result of elevated nitrate loading from the surrounding watershed. Monthly measurements of mean concentrations of dissolved nitrate in the northern basin have indicated an increase from 2.7 μM (in the 1950s) to 9.0 μM (in the 1990s) in the epilimnetic waters and from 2.7 μM (in the 1950s) to 18.7 μM (in the 1990s) in the hypolimnetic waters.

a) Isotopic characteristics of the Lake Biwa–Yodo River watershed. The δ15N of nitrate and the δ13C and δ15N of sedimentary organics have been used as natural tracers for assessing nitrogen and carbon transport in an ecosystem. Sediment δ13C and δ15N values can be also used to study material transport.
from terrestrial to estuarine systems.\textsuperscript{14,15} The nitrate $\delta^{15}$N becomes more positive in the process of denitrification.\textsuperscript{16}

The nitrate $\delta^{15}$N and the $\delta^{13}$C and $\delta^{15}$N of sedimentary organics were measured to elucidate the isotopic biogeochemical structure of the Lake Biwa–Yodo River watershed. It should be emphasized that the $\delta^{13}$C and $\delta^{15}$N of surface sediments, especially $\delta^{15}$N, exhibited the highest value in Lake Biwa within the watershed, as indicated in Fig. 7.\textsuperscript{6}

Nitrogen is supplied to Lake Biwa via precipitation in the surrounding mountain areas. The $\delta^{15}$N of nitorgenous compounds in the precipitation is generally less than 0‰ (Wada and Hattori 1991).\textsuperscript{1}

In fact, the $\delta^{15}$N of the sediments in the Otsuchi watershed in Japan was reportedly lowest at the upper reach, increasing toward the lower reach.\textsuperscript{14}

In an unpolluted watershed like the Otsuchi River in Iwate Prefecture, Japan, most organic matter is produced by terrestrial plants and coastal planktonic algae. The $\delta^{15}$N and $\delta^{13}$C values between these two groups of primary producers are quite different, and a linear relationship is expected for sedimentary organics. However, this relationship is substantially disturbed by the loading of domestic sewage in the Yodo River. The high $\delta^{15}$N value in the middle and lower reaches reflects loading of domestic sewage (ca. 6‰) combined with the denitrification process. The high $\delta^{13}$C value and the low $\delta^{15}$N value in the inner bay areas suggest the rapid growth of red tide algae and the occurrence of nitrogen isotope fractionation during uptake of high concentrations of nitrogenous compounds by algae. This type of isotopic map provides a new avenue for assessing the effects of human activity on the watershed.

The increase in the $\delta^{15}$N value in Lake Biwa is very high (2–8‰ for nitrate and sedimentary organics), whereas the $\delta^{15}$N of introduced nitrogen in the upper mountain areas is between $-2$‰ and 2‰. In nitrogen cycling in natural ecosystems, denitrification and ammonia evaporation are the two main processes that enhance $\delta^{15}$N. Nitrogen loss by ammonia evaporation does not appear to be significant in Lake Biwa, because the waters of the northern basin contain few ammonium ions.\textsuperscript{17}

We recently studied the flows of small streams into the inner bay of Lake Biwa and found that the $\delta^{15}$N of sedimentary organics increased from the upper to the lower reaches of the stream (Hebisuna River; Nishikawa \textit{et al.}, in press\textsuperscript{18}). This finding strongly suggests that denitrification is taking place in the inner bays, together with their small streams, in the Lake Biwa surroundings. Therefore, the higher $\delta^{15}$N value of the sediment and nitrate in Lake Biwa strongly suggests that denitrification is a major contributor to the enhanced $\delta^{15}$N.

\textbf{b) Increase in \(\delta^{15}N\) in Lake Biwa ecosystems during the 20th Century and analysis of the Lake Biwa food web.} The SI ratio of nitrogen ($\delta^{15}$N) could be a useful proxy for delineating lake eutrophication. Nitrogen is not only simply one of the important nutrient elements in a lake, but it is also abundant in anthropogenic sewage and in chemical fertilizers such as ammonium sulfate. Furthermore, the broad range of nitrogen isotope fractionation ratios in lake processes makes these ratios an excellent tracer or monitoring eutrophication.\textsuperscript{19}
In the present study, we measured the $\delta^{15}$N values of muscular tissue from formalin-fixed gobiid fish (isaza) specimens that had been collected in Lake Biwa from as long ago as 1916. Eutrophication of Lake Biwa has worsened during the most recent four decades as a result of anthropogenic perturbations such as increased domestic sewage loading and reduction in the macrophyte area, which plays an important role in water purification.\(^{13,18}\)

In 1916 and 1953, the $\delta^{15}$N values for isaza fish ($\delta^{15}$N\textsubscript{isaza}) were 12.6‰ and 13.3‰, respectively; the values then rapidly increased from the mid-1960s to the 1980s, although some 1970s samples exhibited rather low $\delta^{15}$N\textsubscript{isaza} values (14.4 ± 0.08‰ in 1970, $n = 3$; 14.3‰ in 1972). From 1987 to 1994, $\delta^{15}$N\textsubscript{isaza} varied from 16.7‰ to 15.2‰, showing a near constant or slightly decreasing value.\(^{13}\)

The core nitrogen isotope record ($\delta^{15}$N\textsubscript{sediment}) showed a remarkable increase between 1901 (3.8‰) and 1995 (8.1‰). $\delta^{15}$N\textsubscript{sediment} in the lower portion of the core also increased, starting at 1.1‰ in 1901 and rising at 0.02‰ yr\(^{-1}\) until 1956. In contrast, $\delta^{15}$N\textsubscript{sediment} in the upper portion of the core increased at 0.08‰ yr\(^{-1}\) between 1956 and 1995.\(^{13}\) These results on the $\delta^{13}$C and $\delta^{15}$N of animals, and 40 years of food web structure studies in Lake Biwa are summarized in Fig. 8. Thus, we can use $\delta^{15}$N–$\delta^{13}$C map for assessing human impacts.

c) The $\delta^{15}$N as an index of pollution. Eutrophication of lakes is usually caused by loading of nitrogen and phosphorous-rich organic matter produced \textit{via} domestic sewage. Sewage tends to have a higher $\delta^{15}$N value (ca. 6‰) than that of an unpolluted watershed (3–4‰). As the sewage load increases, $\delta^{15}$N of the watershed as a whole increases from 3‰ to 6‰. If the loading is sufficient to disturb a redox boundary, denitrification will further increase $\delta^{15}$N as shown in Fig. 9a.\(^{18}\)

The $\delta^{15}$N value of particulate organic matter (POM) of sedimentary organics may correlate directly with loading, which, practically speaking, reflects the population density of the areas in question. In the second phase, denitrification might occur with high nitrogen isotope fractionation because the increased loading leads to the appearance of a semi-anerobic zone.\(^{18}\) This phenomenon has been clearly observed for several watersheds in Japan, suggesting that $\delta^{15}$N is an index of eutrophication.\(^{18}\)

A detailed survey was performed to determine the distribution and variation of $\delta^{15}$N values in nitrogenous compounds in a representative small river (Hebisuna River) that flows into Lake Nishinoko, an inner bay of Lake Biwa. A high $\delta^{15}$N value was detected in the lower reaches of the river and the inner bay, most likely due to denitrification. These results strongly suggest that denitrification in small river systems like the Hebisuna watershed has contributed to $^{15}$N enrichment in the Lake Biwa ecosystem during the past...
40 years. We also observed a clear, stepwise, linear correlation between population density and $\delta^{15}N$ values for POM and sediment. These results demonstrate that $\delta^{15}N_{\text{POM}}$ and $\delta^{15}N_{\text{sediment}}$ are helpful indicators for assessing nitrogen loading from domestic sewage. Moreover, they will aid in conceiving new understandings of the environmental capacity of river ecosystems and its relationship to redox conditions. Finally, our data suggest that a population density of 100 to 200 persons/km$^2$ is the upper limit for a watershed in which only simple conventional sewage treatment is in effect (Fig. 9b).

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Profile

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