Japan Geoscience Union Meeting 2015
(May 24th - 28th at Makuhari, Chiba, Japan)

HTT31-10 Room:304 Time:May 27 14:45-15:00

The effects and solution of "mixing problem" in the dietary analysis using stable isotopes

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Dietary analysis using stable isotopes can reveal dietary information in the long timeframe, and it can be applied to ancient animals. These advantages of stable isotope analysis allowed them to be used in the various field of science including anthropology, ecology and archaeology. Especially, stable isotope analysis using dual isotopes; carbon (d^{13}C) and nitrogen (d^{15}N) is the most widely used in every field. Carbon stable isotopes possess distinctly different isotope ratios between C_3 and C_4 plants due to fractionation during photosynthetic carbon fixation. The stable nitrogen isotope ratios increase along with food chain. In this reason, stable isotope analysis using these stable isotopes provides highly reliable dietary information. In addition, recent studies sometimes use stable isotope mixing model, which can evaluated the proportional contribution of each food resource to the diets of individual or groups of target animal.

However, there are several points to be kept in mind when we interpret the result of stable isotope analysis, and the "mixing problem" is one of them. This is attribute to the geometry of sources and mixtures in a mixing diagram, and the problem sometimes diffuses or constrains the possible source contributions. The mixing problem frequently occurs as the number of sources increases (e.g. over 4 sources in dual isotopes). If the geometry of sources and mixtures were likely to involve the mixing problem, the interpretation of stable isotope analysis should be undetermined regardless of the use of mixing model. However, this problem is not sufficiently recognized even in modern times.

In this presentation, we show the effect and solution of the mixing problem using field data of Hokkaido brown bears (*Ursus arctos*). Brown bears are opportunistic omnivore and they consume various diet items including C_3 plants, crops (including C_4 plants), terrestrial animals and salmon. Previous studies showed that the mean d^{13}C and d^{15}N values of C_3 plants, terrestrial animals and salmon of Hokkaido were almost linearly distributed, and the points of Hokkaido brown bears were also mostly plotted on the line. This is exactly the case of mixing problem, and we tried to evaluate the effects of the mixing problem using mixing model analysis and additional use sulfur stable isotopes which can isotopically separate the marine and terrestrial diet.

We collected bone collagen of brown bears in Shiretoko peninsula and their diet items (C_3 herbs, C_3 fruits, corn, terrestrial animals and salmon), and measured carbon, nitrogen and sulfur stable isotope ratios. Then we estimated proportional contribution of each diet items to individual bear's diet using mixing model (SIAR) both in dual (carbon and nitrogen) and triple isotopes (carbon, nitrogen and sulfur), and compared the results of these estimates.

In the results of SIAR using dual isotopes, proportions of each diet items (C_3 herbs, C_3 fruits, corn, terrestrial animals and salmon) were 33.2%, 28.1%, 8.4%, 19.0%, 6.4%, respectively. On the other hand, dietary proportions estimated by triple stable isotopes were 36.5%, 28.3%, 6.8%, 11.4% 10.5%, respectively. Mean absolute differences of proportions of each diet items were highest in terrestrial animals (12.0%). In the dual isotope analysis, the proportion of terrestrial animals tended to be overestimated (max: 53.3%) and salmon and C_3 herbs were likely to underestimated.

Our results showed that the mixing problem practically results in the bias of dietary estimation by stable isotope mixing model. When the geometry of sources and mixtures were likely to involve the mixing problem, the proportions of diet items in the intermediate position of mixing diagram should be overestimated. In this case, interpretation of stable isotope data is difficult, and therefore, addition of other stable isotope elements or discussion about the expected bias should be needed.

Keywords: stable isotope, carbon, nitrogen, sulfur, mixing model, brown bear