

## Analysis of stream food webs using stable nitrogen isotope ratios of amino acids

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Food web studies are a central theme in ecology that relate to material and energy dynamics in an ecosystem. In the stream ecosystem, which connects terrestrial and aquatic ecosystems, stable carbon isotope ratios are sometimes too variable to estimate food sources for organisms. Though stable nitrogen isotope ratios are used to estimate trophic levels (TLs) of organisms, the isotopic enrichment factor per TL is known to be different across various kinds of organisms. For the purpose of better understanding of food web structure in stream ecosystems, a novel technique is indispensable.

Recently, stable nitrogen isotope measurement of individual amino acids (SIAA) has been developed and enabled researches to estimate TLs of organisms in a simple food chain system. In the amino acid metabolism, glutamic acid experiences deamination and transamination, which consequences great isotopic enrichment per TL. On the other hand, phenylalanine conserves its amine during metabolism, resulting in little isotopic enrichment per TL. Therefore, the TLs of organisms in a simple food chain can be determined by following equation (Chikaraishi et al. 2009):

$$TL = (\delta^{15}N_{Glu} - \delta^{15}N_{Phe} + \beta) / 7.6 + 1$$

where  $\delta^{15}N_{Glu}$  and  $\delta^{15}N_{Phe}$  are stable nitrogen isotope ratios of glutamic acid and phenylalanine of an organism, respectively, and  $\beta$  is the nitrogen isotopic difference between phenylalanine and glutamic acid of a primary producer. Since aquatic and terrestrial primary producers have a distinctive  $\beta$  value, a mixing ratio of resources that are derived from both aquatic and terrestrial food chains should be considered to estimate the TLs of organisms in complex food webs. So far, it is still unknown whether the SIAA technique is applicable to understand complex food web structure or not.

In this study, we aimed to analyze stream food webs using the SIAA. In November 2011 and May 2012, we collected stream macroinvertebrates and fishes, together with their potential food sources (periphyton: aquatic primary producer; C3 plant litter: terrestrial primary producer) from upper and lower sites of the two streams, which have contrastive riparian landscapes. Amino acids of organisms and food sources were purified by HCl hydrolysis, followed by Npivaloyl/isopropyl derivation. The SIAA were determined by isotope ratio mass spectrometry coupled to a gas chromatograph via combustion and reduction furnaces.

Stable nitrogen isotope ratios of glutamic acid and phenylalanine identified that the TLs of primary producers (periphyton and C3 plant litter) and primary consumers (e.g., mayfly and caddisfly larva) were 1 and 2, respectively. On the other hand, the TLs (assuming only aquatic food chain) of secondary and tertiary consumers (e.g., stonefly and dragonfly larva and fishes) were lower than those expected by their feeding habits. Since aquatic and terrestrial primary producers have a distinctive  $\beta$  value, we calculated a mixing ratio of both aquatic and terrestrial resources for individual organisms prior to the TL calculation. The estimated TLs (assuming source mixing) of secondary and tertiary consumers roughly corresponded to their feeding habits, suggesting that their diets were derived from both aquatic and terrestrial food chains.

Our result suggests that the SIAA technique is applicable to analyze stream food webs, and perhaps other complex food webs as well. In the presentation, we will add further discussions on the TLs of secondary and tertiary consumers in stream food webs, as well as comparisons of the results among sites, streams, and seasons.

### References

Chikaraishi Y, Ogawa NO, Kashiyama Y, Takano Y, Suga H, Tomitani A, Miyashita H, Kitazato H, Ohkouchi N (2009) Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnology and Oceanography Methods* 7:740-750

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