Res. Org. Geochem. **31**, 29−32 (2015)

Short Article

Isotopic discrimination of $^{15}N/^{14}N$ of amino acids among the calanoid copepod *Acartia steueri* and its food items, eggs, and fecal pellets

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(Received March 20, 2015 Accepted July 17, 2015)

Abstract

Stable nitrogen isotopic composition ($\delta^{15}N$) of glutamic acid and phenylalanine in a laboratory-cultured calanoid copepod *Acartia steueri* and in its food items (the diatom *Thalassiosira weissflogii*), eggs, and fecal pellets was determined to examine the isotopic discrimination of $^{15}N/^{14}N$ of amino acids among these samples associated with a single grazing process. Glutamic acid has a wide variation in the $\delta^{15}N$ value among the bodies (+8.8‰ for 10 days with feeding and +8.9‰ for the following one day without feeding), eggs (+8.8‰), and fecal pellets (+3.3‰) of *A. steueri* and its food items (+0.3‰), whereas phenylalanine falls in a narrow range of the values from −3.7‰ to −3.1‰, $1\sigma=0.3$, for all samples. Based on these $\delta^{15}N$ values, the trophic position ($TP_{Glu/Phe}$) was characterized as 2.1 for bodies and eggs, 1.5 for fecal pellets, and 1.1 for the food items. These $TP_{Glu/Phe}$ values for *A. steueri* and its food items are consistent with the expected trophic positions (2.0 and 1.0, respectively) of these samples. To our knowledge, this is the first published data for the $TP_{Glu/Phe}$ value of fecal pellets, which is 0.4 units higher than that of the food items. Because fecal pellets supply basal food resources for many organisms living in aphotic zones, our data will provide a better understanding of their trophic positions.

Key words: nitrogen isotopic discrimination, trophic position, *Acartia steueri*, fecal pellets

1. Introduction

Isotopic discrimination in $^{15}N/^{14}N$ of amino acids is associated with their metabolisms in the grazing process from diet to consumer species, which allows us to estimate the trophic position of organisms in studied food webs (Chikaraishi et al., 2007). Based on investigations using controlled-feeding experiments and well-characterized wild species, Chikaraishi et al. (2009) established the following equation to calculate the trophic position (TP) of aquatic organisms:

$$TP_{Glu/Phe}=[(\delta^{15}N_{Glu}-\delta^{15}N_{Phe}-3.4)/7.6]+1 \qquad (1)$$

where the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ represent the determined $\delta^{15}N$ values of glutamic acid and phenylalanine, respectively, in a single organism of interest. This is designed with the $TP_{Glu/Phe}$ value corresponding to the actual trophic position of organisms within 0.12 units as an error of accuracy for aquatic food webs. During the last five years, applicability of this equation has been confirmed by several controlled-feeding experiments including for zooplankton (Nakatomi et al., 2013) and by a high-resolution food web study in a natural marine ecosystem (Chikaraishi et al., 2014).

However, the trophic position estimates based on the $\delta^{15}N$ values of amino acids are still in the early development stage, because the universality of the isotopic discrimination is mostly unknown among many organisms. For example, Dale et al. (2011) reported that the $TP_{Glu/Phe}$ values for brown stingray were underestimated by approximately one unit compared with the trophic position independently estimated by stomach content analysis. Moreover, Germain et al. (2013) illustrated a significant compression (i.e., 7.6‰ to 4.3‰) of the isotopic discrimination for harbor seal. Thus the trophic position estimates based on the $\delta^{15}N$ values of amino acids clearly need further evaluation based particularly on controlled-feeding experiments for various species, conditions, and trophic positions.

Copepods usually occupy approximately more than 70% of net-collected plankton biomass as one of the most abundant zooplankton species in the marine ecosystem (Lalli and Parsons 1997). They play an essential role in aquatic ecosystems as the first linkage between phytoplankton and higher trophic position consumers such as fish (Runge, 1988; Mauchline, 1998),

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where the solar energy fixed by phytoplankton moves along food chains through zooplankton. Moreover, the fecal pellets produced by copepods contribute considerably to global carbon and nutrient cycles, such as by fixing photosynthetically produced organic materials for vertical transportation from the euphotic surface ocean to deep sea column layers and the sea floor (Angel, 1984; Noji, 1991). These sinking fecal pellets supply basal food resources for many organisms living in the water column, particularly food webs of aphotic zones (Wiebe et al., 1976; Turner, 1977).

In this study, we determined the stable nitrogen isotopic composition (δ^{15}N) of glutamic acid and phenylalanine in a laboratory-cultured calanoid copepod *Acartia steueri* and in its food items (the diatom *Thalassiosira weissflogii*), eggs, and fecal pellets. It is previously reported that the isotopic discrimination on glutamic acid and phenylalanine in *A. steueri* cultured with haptophyte *Isochrysis galbana* for 6 days was +8.3±0.5‰ and +0.3±0.2‰, respectively (they are very close to the standard values: +8.0±1.2‰ and +0.4±0.5‰, respectively, Chikaraishi et al., 2009), and that no substantial difference in the δ^{15}N value is found between male and female bodies as well as female's reproductive eggs (Nakatomi et al. 2013). In this study, we (1) further clarify if isotopic discrimination depends on the food items (haptophyte *vs.* diatom) and (2) newly reveal the isotopic discrimination and the apparent trophic position (the $TP_{Glu/Phe}$ value) of fecal pellets. To our knowledge, the first published data for the $TP_{Glu/Phe}$ value of fecal pellets are reported.

2. Samples and methods

The calanoid copepod *A. steueri* was collected using a plankton net (diameter: 30 cm; mesh size: 180 μm) at Manazuru Port, Sagami Bay, Japan (35°09′49″ N, 139°10′33″ E) in October 2014. Fifty adult females were sorted under a dissecting microscope and then incubated for 11 days (21ºC, a cycle of 12 h interval of light and dark) in 500 mL of 0.22 μm filtered seawater from collection site. The diatom *T. weissflogii* cultured with f/2 media was given *to* the *A. steueri* as a sole source of food items at 1.0 mg/L for 10 days with feeding and for the following one day without feeding to minimize their gut contents. Seawater was changed every day during incubation. Eggs and fecal pellets from days 8 to 10 were gently collected on 20 μm mesh and placed in 6 holes multi-well dishes. They were picked up with a Pasteur pipette under a dissecting microscope after rinsed with 0.22 μm filtered seawater.

Twenty-one and eight females of *A. steueri* were collected on days 10 and 11, respectively. Those samples were immediately fixed with 1% buffered formalin at room temperature. Five samples, eggs (combined approximately 900 eggs produced for the three days), fecal pellets (combined with those for the three days), the bodies of 21 females at day 10 and of 8 females at day 11, and the food items *T. weissflogii* were used in this study.

These samples were prepared for stable nitrogen isotope analysis of amino acids, after HCl hydrolysis and *N*-pivaloyl/isopropyl (Pv/iPr) derivatization, according to the procedure in Chikaraishi et al. (2009). The isotopic composition was determined by gas chromatography/isotope ratio mass spectrometry (GC/IRMS) using a 6890N GC (Agilent Technologies) instrument coupled with a Delta^{plus}XP IRMS instrument through combustion (950ºC) and reduction (550ºC) furnaces *via* a GC-C/TC III interface (Thermo Fisher Scientific). The isotopic composition was expressed relative to atmospheric nitrogen (δ^{15}N, ‰ vs. AIR) on a scale normalized to the known δ^{15}N values of nine isotopic reference amino acids (from −26.1‰ to +45.7‰, Indiana University and SI science co., Sato et al., 2014). The accuracy and precision for the isotope measurements of the reference amino acids were 0.0‰ (mean of Δ) and 0.34‰ (mean of 1σ), respectively. The $TP_{Glu/Phe}$ values were calculated using equation (1).

3. Results and discussion

3.1. Stable nitrogen isotopic discrimination of amino acids and estimated trophic position

The δ^{15}N values of glutamic acid are +8.8‰, +8.9‰, +8.8‰, +3.3‰, and +0.3‰, and those of phenylalanine are −3.2‰, −3.1‰, −3.2‰, −3.6‰, and −3.7‰ for the bodies of females at day 10, those at day 11, eggs, fecal pellets, and food items, respectively (Fig. 1). According to the analytical error in the δ^{15}N value in this study (1σ=0.34‰), a variation in the δ^{15}N value of glutamic acid (from +0.3‰ to +8.9‰) is significantly large among these samples within a controlled-feeding experiment, whereas that of phenylalanine (from −3.7‰ to −3.1‰) is substantially small or negligible among them. Based on these δ^{15}N values and equation (1), the $TP_{Glu/Phe}$ values are calculated to be 2.1, 2.1, 2.1, 1.5, and 1.1 for the bodies at days 10 and 11, eggs, fecal pellets, and food items, respectively.

3.2. Isotopic discrimination in ^{15}N/^{14}N between *A. steueri* and food items

According to previous findings on the isotopic discrimination of amino acids in a grazing process, glutamic acid has a significant enrichment in ^{15}N by 8.0±1.2‰, whereas phenylalanine has a little enrichment in ^{15}N by 0.4±0.5‰ during each shift in trophic position (Chikaraishi et al., 2009). This is

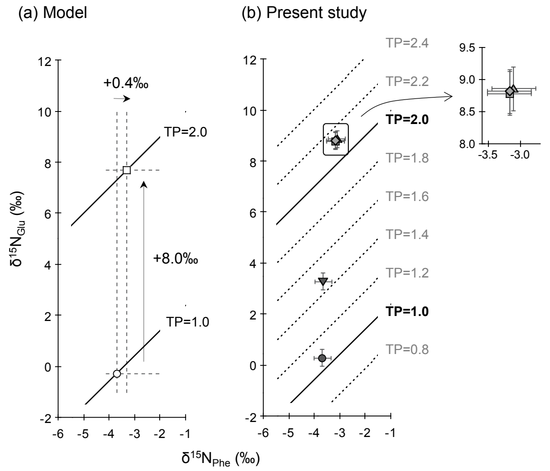

Fig. 1. Cross-plots of the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values: (a) a standard model for the isotopic discrimination in $^{15}N/^{14}N$ in a consumer (open square) feeding only on a primary producer (open circle) (after Chikaraishi et al., 2009) and (b) data observed in this study for bodies at days 10 and 11 (filled square and triangle, respectively), eggs (filled diamond), fecal pellets (filled reversed triangle) of *A. steueri*, and food items (filled circle), with 0.34‰ error bars as the analytical precision. Solid and dashed lines indicate the trophic isocline for integer-based numbers (1.0 and 2.0) and 0.2 intervals of the trophic position, respectively, based on the equation (1).

illustrated by how, on the cross plot for the $\delta^{15}N$ values of glutamic acid and phenylalanine, consumer and resource species would be plotted on lines of the integer-based number of trophic positions (2.0 and 1.0, respectively) with enrichment in ^{15}N by 8.0‰ for glutamic acid and by 0.4‰ for phenylalanine from resource to consumer species, if the consumers feed only on uniform primary producers as their food items (Fig. 1a).

In this study, although the plots of the bodies and eggs of *A. steueri* and of its food items seem to be slightly, positively shifted from the lines of TP = 1.0 and 2.0, respectively (Fig. 1b), these data are likely to be consistent with the standard model according to both errors in the isotope analysis of this study (i.e., 0.34‰) and trophic position estimates in this approach (i.e., 0.12 unit, Chikaraishi et al., 2009). Indeed, the isotopic discrimination between consumer and resources species is +8.5‰, +8.6‰, and +8.5‰ for glutamic acid and +0.5‰, +0.6‰, and +0.5‰ for phenylalanine in the bodies at days 10 and 11 and in the eggs, respectively. No substantial differences are thus found among these three samples. Based on these results, we conclude (1) that gut contents remained can contribute little to the isotopic discrimination in amino acids of *A. steueri*, (2) that the isotopic discrimination is consistent with the general values reported in the previous study (Chikaraishi et al., 2009) and in a previous controlled-feeding experiment of *A. steueri* with a different algal species, the haptophyte *I. galbana* (Nakatomi et al., 2013), and (3) that the effects of food items (haptophyte *vs*. diatom) and egg production on the isotopic discrimination of *A. steueri* are very small or almost negligible.

3.3. Isotopic discrimination in $^{15}N/^{14}N$ on fecal pellets

The $\delta^{15}N$ values of fecal pellets are +3.3‰ and −3.6‰ for glutamic acid and phenylalanine, respectively, which are plotted into an intermediate zone between bodies and the food items of *A. steueri*. The $TP_{Glu/Phe}$ value of fecal pellets from *A. steueri* was calculated as 1.5 in Fig. 1b, which was characterized as 0.4 units higher than the 1.1 for food items. These results may indirectly reflect the microbial activities on the food-derived amino acids through the gut of *A. steueri* and/or in the fecal pellets, but this assumption is not yet confirmed. Although we have only one set of data in this study, it is a value integrated for a large number of fecal pellets from 50 individuals of *A. steueri* within the experiment. Therefore, these data may be tentatively available in the discussion of the isotopic discrimination and the $TP_{Glu/Phe}$ value (apparent trophic position) of fecal pellets.

To our knowledge, this is the first published data for the $TP_{Glu/Phe}$ value of fecal pellets. However, this $TP_{Glu/Phe}$ value is well consistent with the expected value of marine snow, 1.4 ± 0.1, in the western north Pacific (Miller et al., 2012) and with the determined value of sediment traps (450 m depth), 1.4, in the Santa Barbara basin (Batista et al., 2014). Based on the data in this study and several previous studies, we predict that the $TP_{Glu/Phe}$ value of fecal pellets would be approximately 0.4 units higher than that of the food items and that these phenomena may be universal in many cases of aquatic environments.

Fecal pellets supply basal food resources for many organisms living in aphotic zones. It may also be a significant issue that the isotopic composition of amino acids is potentially modified by microbial activities during the vertical transportation from the euphotic surface ocean to deep sea column layers. Thus our data may provide a better understanding of the trophic position of organisms living in aphotic zones. It appears that food chains in aphotic zones and the sea floor start from the trophic position of 1.4–1.5 or more, unlike the 1.0 in euphotic zones of the ocean.

Acknowledgments

We thank two anonymous reviewers and Dr. O. Seki (associate editor) of Hokkaido University for their valuable suggestions and thoughtful comments that were very helpful for improving the manuscript. We also thank Dr. S. Shimode of the Manazuru Marine Laboratory, Yokohama National University, for his assistance with sample collection.

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