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Articles

Difference in the foraging strategy between daphnia and copepods in Shirakoma-ike: evidence from ^{15}N / ^{14}N of amino acids

Atsushi Urai[*, **, a] and Ho-Dong Park[***]

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Abstract

Trophic position (TP) of the daphnia *Daphnia longispina* and the copepod *Acanthodiaptomus pacificus* in an oligotrophic lake, Shirakoma-ike, was investigated via stable nitrogen isotope composition (^{15}N/^{14}N) analysis of amino acids, to understand the foraging strategy of these two dominant freshwater zooplankton species in an oligotrophic lake. The surface water of this lake is frozen in winter, and the life cycle and trophic behavior are different between these two species: the daphnia is found only in spring-autumn, whereas the copepod is found in whole season. In the present study, we found that the TP is 2.1 ± 0.0 for the daphnia in spring-autumn and 2.3 ± 0.3 for the copepod in whole season. These results reveal strong herbivory for the daphnia compared to dietary plasticity and facultative omnivory for the copepod. The latter is suggested to feed on phytoplankton for spring and autumn (TP=2.1 ± 0.0) and on both phytoplankton and heterotrophic microbes (e.g., protists and bacteria) for summer and winter (TP=2.6 ± 0.0). The foraging strategy is thus different between daphnia and copepods in this lake. This finding may explain why the daphnia is absent whereas the copepod is present in the frozen lake in winter where primary production is limited.

1. Introduction

Daphnia and copepods are two of the most dominant zooplankton species in freshwater environments such as ponds and lakes (Wetzel, 2001; Williamson, 1983, 1986; Ebert, 2005). They play a fundamental role in freshwater ecosystems, as a primary carrier of the solar energy fixed by phytoplankton into food webs (e.g., Sarvala and Halsinaho, 1990). However, it is well known that the biomass of zooplankton frequently exceeds that of phytoplankton even after spring and autumn blooms of phytoplankton in lakes particularly for temperate and sub-polar regions, although the production should be exponentially decreased along food chain (e.g., Sommer, 1989; Hairston et al., 1960). Moreover, it has been reported that such zooplankton biomass/density dynamics is species-specific, and is independent of phytoplankton biomass in the environments (e.g., Sommer et al., 2003). Allochthonous materials (e.g., plant leaves and their detritus) thus have been also suggested as a potentially food source for zooplankton in oligotrophic lakes (e.g., Janson et al., 2000; Karlsson et al., 2003; Cole et al., 2011).

Shirakoma-ike is a representative oligotrophic lake where such significant unbalance of plankton community was reported, as (1) daphnia and copepods are the dominant zooplankton species that have apparently more biomass than phytoplankton, and (2) the daphnia is found only in spring-autumn, whereas the copepod is found in whole season (e.g., Kadota, 1960; Lee et al., 2002). A number of previous studies have investigated potential food sources of these zooplankton in this lake, to solve the paradox on the plankton community in freshwater environments (e.g., Kadota, 1960; Lee et al., 2002). For instance, based on gut content analysis, Kadota (1960) first identified attached-algae, bacteria, and detritus as potential food sources for the zooplankton species. More recently, Lee et al. (2002) suggested a large difference in the food sources between daphnia and copepods, based on the stable carbon and nitrogen isotopic compositions of bulk tissues for daphnia (δ^{13}C = −29.6 ± 0.9‰ and δ^{15}N = +1.3 ± 0.4 ‰, respectively) and copepods (δ^{13}C = − 34.9 ± 1.2‰ and δ^{15}N = −0.4 ± 0.9‰, respectively). However, the identification of food sources and their contri-

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[*] Department of Science and Technology, Graduate School of Medicine, Science and Technology, Shinshu University, 3-1-1 Asahi, Matsumoto, 390-8621, Japan

[**] Biogeochemistry Program (BGC), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka, 237-0061, Japan

[****] Department of Environmental Sciences, Faculty of Science, Shinshu University, 3-1-1 Asahi, Matsumoto, 390-8621, Japan

[a] Corresponding author. e-mail: uraia@jamstec.go.jp (Atsushi Urai)

bution to the zooplankton species have been poorly understood so far.

Food web studies, particularly for the estimation of trophic position (TP) of organisms in food webs, have advanced remarkably during the last decade, by the use of stable nitrogen isotopic composition analysis of amino acids (e.g., Chikaraishi et al., 2007; McCarthy et al., 2007: Popp et al., 2007). This methodology has been successfully applied marine and freshwater environments (e.g., Kruse et al., 2015; Hirahara et al., 2015; Ohkouchi et al., 2015; Kruger et al., 2016). The TP has been simply calculated using the δ^{15}N values of glutamic acid ($\delta^{15}N_{Glu}$) and phenylalanine ($\delta^{15}N_{Phe}$), with the following equation (1) (Fig.1, Chikaraishi et al., 2009, 2014):

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

whereβ represents the difference between $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values in primary producers (−3.4‰ for algae and cyanobacteria, +8.4 ‰ for plant leaves). It is known that the error of TP estimates (within 0.2 unit, Chikaraishi et al., 2009) is better than that in the traditional ones (e.g., gut content analysis, δ^{13}C and δ^{15}N analysis of bulk tissues, etc.), although major factors controlling for the δ^{15}N values of amino acids and the potential uncertainty of this methodology have still been debated (e.g., McMahon and McCarthy, 2016; Ohkouchi et al., 2017; Takizawa et al., 2017; Choi et al., 2018). Moreover, the contribution from aquatic and terrestrial primary producers (e.g., algae vs. plant) to food webs has been evaluated by applying mixing models with the $\delta^{15}N_{Phe}$ value (e.g., Naito et al., 2010, 2015).

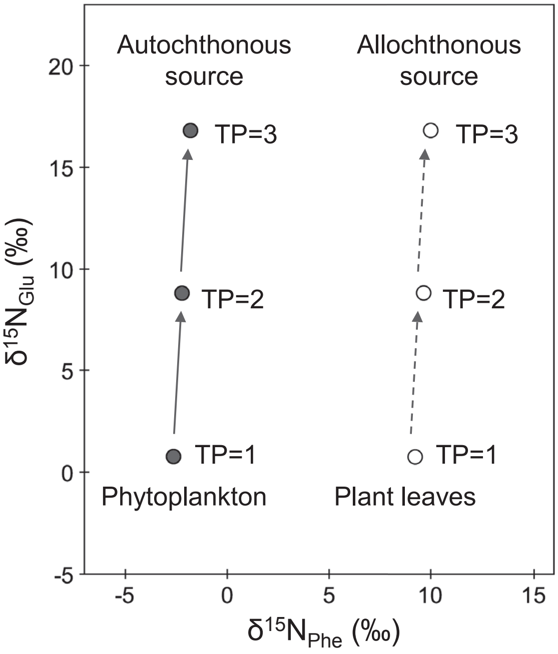

Fig. 1. Schematic illustrations of the trophic position (TP) of autochthonous and allochthonous source based on δ^{15}N values of Phe and Glu.

In this study, we apply this methodology (δ^{15}N of amino acids) to estimate the TP and the potential food sources (and its seasonal variation) of the daphnia *Daphnia longispina* and the copepod *Acanthodiaptomus pacificus* in Shirakoma-ike. We further evaluate the foraging strategy of these two dominant freshwater zooplankton species in the oligotrophic lake.

2. Methods

Shirakoma-ike is a subalpine oligotrophic-dystrophic lake, located in Nagano prefecture, Japan (36° 03'5.1N, 138°21'43.2E, Fig.2). The altitude and surface area of the lake are 2,115 m and 0.11 km^2, respectively. The lake has no permanent input and output flowing with a maximum water depth of 8.6 m, and surface water freezes over winter (from the middle of November to May). The pH of the lake water is approximately 5, making no fish habitable in the lake. Phytoplankton can bloom in spring and autumn, but the concentration of chlorophyll *a* is lower than 2μg/L even for blooming periods and further decreased to 0.5 μg/L for winter (Table 1). The daphnia *Daphnia longispina* and the copepod *Acanthodiaptomus pacificus* are dominant zooplankton species, and the daphnia is found only in spring-autumn (20-120 × 10^3 individuals/m^2), whereas the copepod is found in whole season (50-300 × 10^3 individuals/m^2) (Table 1). Water mites, phantom midge, and dragonfly larva may be high TP omnivores or carnivores in this lake (Lee et al., 2002).

The daphnia and the copepod were collected from around center of the lake in spring (June), summer (August), autumn (November), and winter (December, but *A. pacificus* only because absence of *D. longispina*) in 2015. These two species were sorted under a dissecting microscope, freeze dried, and kept at room temperature until the isotope analysis. According to no substantial contribution of gut contents to the isotope analysis (e.g., Hirahara et al., 2015), the gut content of these species was not eliminated before analysis.

The dried samples (approximately 1.0 mg) were prepared for stable nitrogen isotopic composition analysis of amino acids, after HCl hydrolysis and *N*-pivaloyl/isopropyl (Pv/OiPr) 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

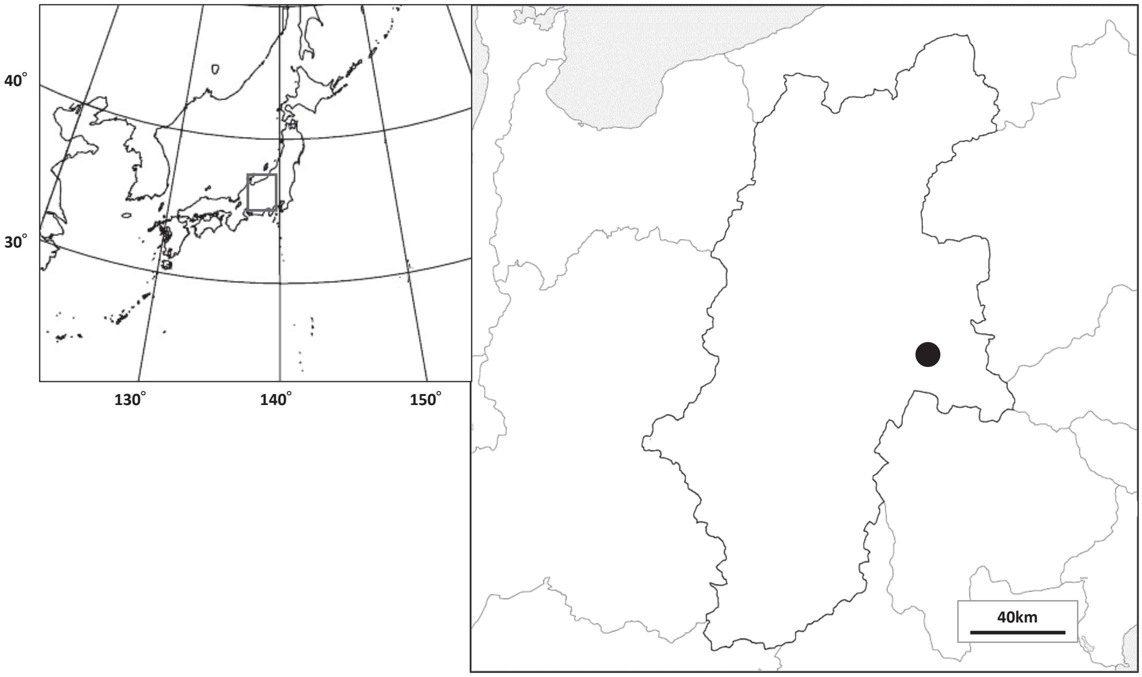

Fig. 2. Geological location of sampling site at Nagano pref., Japan.

Table1. Seasonal change in Chlorophyll a and density of zooplankton in Shirakoma-ike.

	Unit	2011								2012						
		May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Mar	Apr	May	Jun	Jul	Aug
Chl.a	μg/L	0.79	1.83	1.38	1.83	1.47	1.81	1.29	0.86	0.47	0.23	0.26	1.23	0.88	1.34	1.49
Zooplakton density																
D.longispina	$\times 10^3$ ind/m^2	0.3	28.0	93.7	45.8	40.3	30.7	11.7	1.5	0.0	0.0	0.0	15.0	39.4	114.9	32.3
A.pacificus	$\times 10^3$ ind/m^2	121.2	154.3	125.8	89.7	73.5	54.5	46.8	58.2	51.2	44.4	55.0	104.2	294.2	97.9	95.9
Rate of having egg																
D.longispina	%	0.0	16.0	1.1	1.6	39.2	12.3	3.2	0.0	0.0	0.0	0.0	0.0	13.8	22.7	52.1
A.pacificus	%	0.0	1.8	3.0	0.0	13.0	10.7	63.4	60.5	65.2	56.9	20.5	11.1	8.7	8.3	0.0

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 ($\delta^{15}N$, ‰ vs. AIR) on a scale normalized to the known $\delta^{15}N$ values of isotope 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 0.5‰ (mean of 1σ), respectively. The TP was calculated using the equation (1), with $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ for each sample, which determined in the present study, and with − 3.4 ‰ for the β value (Chikaraishi et al., 2009).

3. Results and discussion

3.1. The $\delta^{15}N$ values and the estimated TP

A little change in the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values was found in daphnia (i.e., from +6.2 to +8.0‰ and from −5.4 to −4.1‰, respectively) in spring-autumn, resulting in no substantial change in the estimated TP (2.1 ± 0.0) through season (Table 2, Fig.3a). On the other hand, a gradual increase in the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values

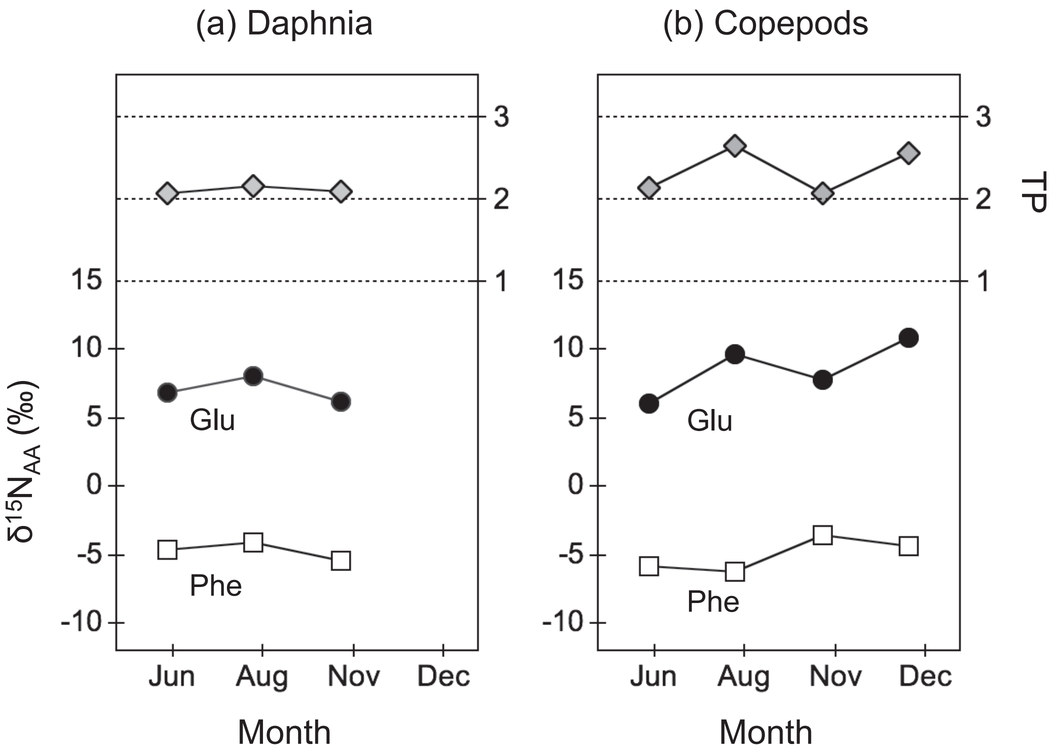

Fig. 3. The $\delta^{15}N_{Glu}$ (filled circle) and $\delta^{15}N_{Phe}$ values (open square), and TP (gray diamond) of daphnia and copepods in Sirakoma-ike.

Table2. Nitrogen isotopic composition of amino acids in *A.pacificus* and *D. longispina*.

	Collection date	$\delta^{15}N$ (‰, vs Air)							$TP_{Glu/Phe}$ ※
		Alanine	Glycine	Valine	Leucine	Isoluecine	Glutamic acid	Phenyl alanine	
A.pacificus	Jun.	3.2	-13.5	4.5	-2.7	0.0	6.0	-5.9	2.1
	Aug.	9.6	-5.1	6.9	3.1	3.3	9.6	-6.2	2.6
	Nov.	5.5	-10.6	6.6	-1.2	0.7	7.8	-3.6	2.1
	Dec.	12.5	-3.2	8.3	3.1	5.6	10.8	-4.4	2.6
D.longispina	Jun.	3.2	-10.4	5.8	0.9	3.2	6.8	-4.7	2.1
	Aug.	n.d.	-4.0	11.8	5.6	7.9	8.0	-4.1	2.1
	Nov.	3.2	-12.3	3.5	1.1	1.2	6.2	-5.4	2.1

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

was apparently found in copepods (from +6.0 to +10.8‰ and −6.2 to −3.6‰, respectively) from spring to winter (Table 2, Fig.3b). Moreover, a zigzag change in the TP was obtained in copepods, as the TP for spring and autumn (TP=2.1, n=2) is lower than that for summer and winter (TP=2.6, n=2). Thus, the TP of zooplankton indicates the foraging strategy specific to species, which may be related to the absence of daphnia vs. the presence of copepods in winter (i.e., limited primary production in the freezing period) of the lake (see below).

3.2. Foraging strategy

It is known that the $\delta^{15}N_{Phe}$ values in consumers principally reflects an integrated value for that of basal recourses of the consumers in food webs. Furthermore, the $\delta^{15}N_{Phe}$ values of autochthonous sources (e.g., phytoplankton) are much lower (by ~11.8‰) than those of allochthonous inputs (e.g., plant leaves) (e.g., Chikaraishi et al., 2014; Ohkouchi et al., 2017). According to this knowledge, overlapping in the $\delta^{15}N_{Phe}$ range between daphnia (from − 5.4 to − 4.1 ‰) and copepods (from −6.2 to −3.6‰) observed in this study (Table 2) clearly indicates that these two zooplankton species belong to the same food web in this lake. Also, these low $\delta^{15}N_{Phe}$ values and their small variation suggest little or negligible input from allochthonous food sources to these zooplankton species in this lake.

On the other hand, a difference in the trend of TP through season (Fig.3) reveals distinct foraging strategy for these two zooplankton species in this lake. Based on the potential estimation error in the TP (i.e., 0.2 units, Chikaraishi et al., 2009), the TP for the daphnia (2.1 ± 0.0) and the copepods (2.3 ± 0.3) indicates that they contribute mainly as herbivorous and omnivorous zooplankton, respectively, in the food webs of this lake. Moreover, such herbivory for the daphnia did not change through season. Beside the daphnia, the degree of omnivory for the copepods is variable and shows difference between seasons as more herbivory (TP=2.1, n=2) that feeds on phytoplankton for spring and autumn than omnivory (TP=2.6, n=2) that feeds on both phytoplankton and heterotrophic microbes (e.g., protists and bacteria) for summer and winter. Thus, these results reveal strong herbivory for the daphnia compared to dietary plasticity and facultative omnivory for the copepod in this lake.

In the observation, the population size of daphnia (20-120 × 10^3 individuals/m^2) was much smaller than that of copepods (50-300 × 10^3 individuals/m^2) in this lake. Moreover, the life cycle of daphnia is somewhat different from that of copepods: daphnia produce resting eggs (or sometimes called winter eggs) for over-wintering, whereas copepods can survive winter even in adult stages (e.g., Carvalho and Wolf, 1989, Wolf and Carvalho, 1989). Although, based on the TP and the $\delta^{15}N_{Phe}$ values, we cannot fully explain these differences in the population and life cycle between the two zooplankton species, we predict the following foraging strategy and life cycle:

Daphnia: They feed predominantly on phytoplankton in spring-autumn, even under the strong limitation of the phytoplankton biomass, particularly for summer. They, however, produce resting eggs, for adapting the very strong limitation of phytoplankton biomass in the freezing period.

Copepods: Like daphnia, they feed preferentially on phytoplankton in spring and autumn. However, their diet is shifted from phytoplankton other food sources – probably heterotrophic microbes such as protists and bacteria – in summer and winter, because copepods production cannot be supported only from the phytoplankton biomass.

There is major paradox that these zooplankton species have more biomass than phytoplankton in this lake, and therefore a number of previous studies have investigated potential diet sources (instead of phytoplankton) for these zooplankton (e.g., Kadota, 1960; Lee et al., 2002). However, based on the results of this study, (1) the TP for the daphnia (2.1 ± 0.0) and the copepods (2.3 ± 0.3) and (2) the low $\delta^{15}N_{Phe}$ values and their small variation (from −6.2 to −3.6‰) suggest that these two zooplankton species are mainly supported by the phytoplankton production, although the concentration of chlorophyll *a* determined is only 1.5μg/L during the open ice season. In the field observation, short (within a few days) bloom of phytoplankton is frequently appeared after temporal inputs of snow-malting-water and/or rainwater. The short bloom of phytoplankton may partially support the biomass of these zooplankton species.

Heterotrophic microbes (e.g., protists and bacteria) potentially have the TP close to 1.0 (as phytoplankton), because they *de novo* synthesize amino acids from carbon sources and ammonia (Yamaguchi et al., 2017), and may supply autochthonous food sources to these zooplankton species in freshwater lake (e.g., Janson et al., 2000; Karlsson et al., 2003; Cole et al., 2011). However, we assume that bioavailable carbon sources (e.g., glucose, boiled starch, but not starch, cellulose, lignin, etc.) are very limited or absent in the lake if allochthonous (e.g., plant leaves) inputs are significantly large. In this study, although we cannot accurately estimate the contribution of such microbes to the TP=1 organisms, we predict that phytoplankton is major sources to support the biomass of these two zooplankton species.

3.3 Potential uncertainty in the TP estimate

Applying this new methodology, we should consider the universality on the TP estimation for the zooplankton species. Chikaraishi et al. (2009) first established the equation (1) for marine zooplankton and fish, based on large and small trophic enrichment $^{15}N_{Glu}$ and $^{15}N_{Phe}$, respectively. Moreover, the applicability of this equation (1) has been confirmed in diverse organisms including fungi, bacteria, insects, fish, and mammals (e.g., Steffan et al., 2015; Yamaguchi et al., 2017). However, trophic elevation in the $\delta^{15}N_{Glu}$ value may vary unique to species and/or among growth conditions such as the quality of diets (Chikaraishi et al., 2015; McMahon et al., 2015; McMahon and McCarthy, 2016). Little trophic elevation in the $\delta^{15}N_{Glu}$ value was reported in protozoan (Gutoérrez-Rodríguez et al., 2014) and protistan (microzooplankton) (Décima et al., 2017). More recently, Choi et al. (2018) reported that the TP of herbivorous gastropod estimated is lower than 2.0, because of metabolic flux of amino acids unique to these species. Thus, trophic elevation in the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values is universal in many species, but is not always in all species including several zooplankton. Based on these findings, we suggest that further studies are required for the estimation of accurate TP of zooplankton with respect to the metabolic flux of amino acids in each species.

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