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Factors for controlling stable isotopic composition of amino acids of marine organisms: Implication to aquatic ecosystem studies

（海洋生物に含まれるアミノ酸の安定同位体比を変化させる要因の解明）

Daochao Xing

Abstract of Ph.D thesis

16 September, 2021

1. Introduction

Marine ecosystems have been damaged by human activities, including the extinction of organisms by overfishing, the concentration of pollutants by biomagnification, and the acidification of ocean by CO_2 emission. For the Ph.D study, we mainly focus on biomagnification and ocean acidification. Trophic position (TP) of organisms is a factor for determining biomagnification. However, there is an issue on the determination in previous studies, because calculation of the TP of organisms is challenging. Also, energy consumption of organisms is a factor for estimating the effect of ocean acidification. However, there is an issue on the estimation, because evaluation of the energy consumption is challenging. Compound-specific isotope analysis of amino acids (CSIA-AA) is one of potential powerful tools for solving these two issues through this analysis allows us accurately to calculate TP and evaluate energy consumption of organisms in environments.

To solve these two issues, the Ph.D study is composed of two studies, 1) calculating the accurate TP of organisms in food webs, and 2) evaluating the energy consumption of organisms in ocean acidification. For the former, we demonstrated the applicability of CSIA-AA for coastal marine fish in a complex environment (where has isotopically-distinct multiple nitrogen sources), clarified the variation in the $\delta^{15}N$ value of amino acids among tissue types within in a single organism, and illustrated a spatial variation in the $\delta^{15}N$ value of primary producers in the complex environment. For the latter, we evaluated the energy consumption of marine organisms in laboratory aquarium system with a constant pH ranging from 7.3 to 8.1.

2. Methods

2.1 Calculating trophic position of organisms and baseline $\delta^{15}N$ of food webs

The trophic position (TP) of organisms were calculated based on the comparison of large (8.0‰) and small (0.4‰) trophic discrimination factors (TDF) between glutamic acid and phenylalanine, respectively, according to the equation (1) reported in Chikaraishi et al. (2009):

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

where $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ represent the $\delta^{15}N$ values of glutamic acid and phenylalanine, respectively, in a studied organism, β represents a constant offset between the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values found in primary producers (e.g., 3.4‰, Chikaraishi et al. 2009).

The baseline $\delta^{15}N$ of food webs were calculated based on the elimination of small TDF on the $\delta^{15}N$ values of phenylalanine in studied organisms, according to the equation (2):

$$\delta^{15}N_{Baseline,Phe} = \delta^{15}N_{Phe} - 0.4\ (TP - 1) \qquad (2)$$

where $\delta^{15}N_{Baseline,Phe}$ represents the weighted-mean $\delta^{15}N$ value of phenylalanine in primary producers for month to year scale in a studied food web.

2.2 Evaluating energy consumption of organisms

The energy consumption of organisms was evaluated based on changes in the $\delta^{15}N$ value of amino acids of organisms between experimental and control conditions, according to the following equation (3):

$$\Delta\delta^{15}N = \delta^{15}N_{Experimental} - \delta^{15}N_{Control} \qquad (3)$$

where $\delta^{15}N_{Experimental}$ and $\delta^{15}N_{Control}$ represent the $\delta^{15}N$ values of an amino acid in organisms that reared in experimental and control conditions, respectively. According to the Rayleigh fractionation model, the $\Delta\delta^{15}N$ values are positively corelated with the flux (F) of amino acid metabolism in organisms, which approximately represents energy consumption of organisms, as the following equations (4):

$$\Delta\delta^{15}N = 1000 \times [F_{Experimental}{}^{(\alpha - 1)} - F_{Control}{}^{(\alpha - 1)}] \qquad (4)$$

where α represents the isotopic fractionation factor associate with amino acid metabolism in organisms. Based on the equation (4), positive and negative $\Delta\delta^{15}N$ values can be explained by that organisms consume much and less energy, respectively, in these experimental conditions.

Study 1: Calculating the accurate TP of organisms in food webs

The objectives of study 1 is to demonstrate the applicability of CSIA-AA for calculating TP of organisms in a complex ecosystem where has isotopically-distinct multiple nitrogen sources. This is mainly because, since 2009, CSIA-AA has been used mainly for ecosystems where have single nitrogen source. The study 1 is composed of 3 sub-studies:

1.1 **Charactering food web structure for fish communities**

We applied CSIA-AA to calculate TP and the $\delta^{15}N_{Baseline,Phe}$ values for 13 fish and 1 squid species collected from a coastal area of Sagami Bay, Japan, where has a large diversity in the isotopic baseline. Our results indicate that the TP of fish and squid varies between 2.9 and 3.9 (i.e., omnivorous, carnivorous, and tertiary consumers), with a small variation within individual species (Fig. 1). Moreover, the $\delta^{15}N$ values of phenylalanine revealed a large diversity in the isotopic baselines between and within species. Low (7.8-10.3‰) and high values (18.6−19.2‰), with a small variation ($1\sigma <$ 1.0‰), were found in the 2 offshore species and 3 coastal species, respectively. In contrast, highly variable values (9.8-19.7‰), with a large variation within species ($1\sigma >$ 1.0‰), were found for the 9 migratory species. These results reveal that there is differential trophic exploitation of habitats between offshore and coastal species, particularly among individual specimens for the migratory species, even though these species were collected in a single area of Sagami Bay.

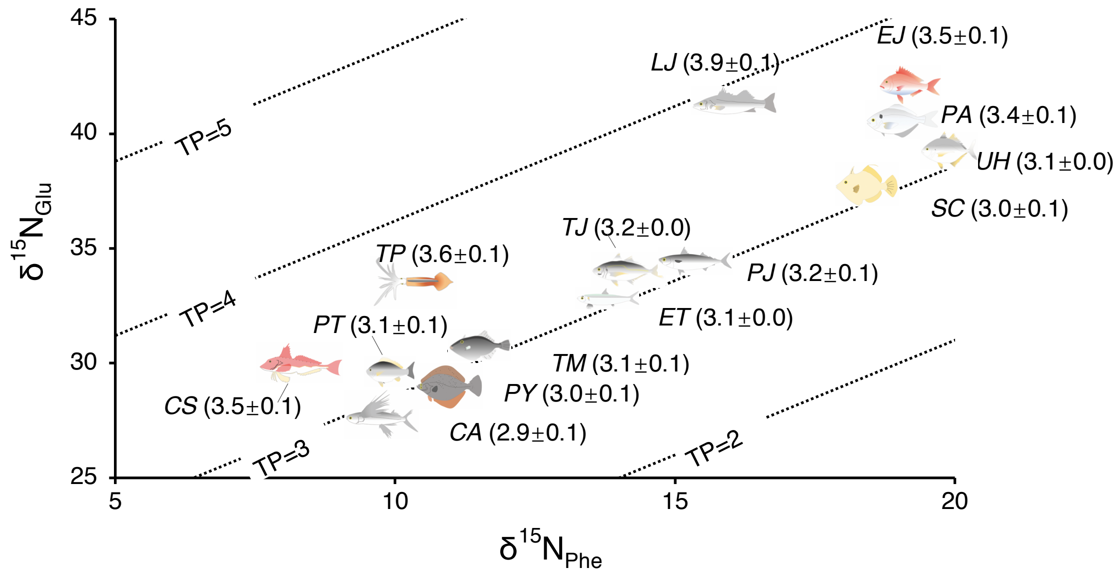

Fig. 1. Cross-plot of the δ^{15}N values of glutamic acid and phenylalanine. Lines represent integer TP (i.e., 2, 3, 4, and 5) estimated with equation (1). Numbers in brackets show the mean ± SD of TP for the species. CA: *Cypselurus agoo*, EJ: *Evynnis japonica*, ET: *Etrumeus teres*, LJ: *Lateolabrax japonicus*, PA: *Psenopsis anomala*, PJ: *Pneumatophorus japonicus*, PT: *Parapristipoma trilineatum*, PY: *Pseudopleuronectes yokohamae*, SC: *Stephanolepis cirrhifer*, TJ: *Trachurus japonicus*, TM: *Thamnaconus modestus*, TP: *Todarodes pacificus*, UH: *Uraspis helvola.*

1.2 **Comparing δ^{15}N values of amino acids between tissue types**

We compared the tissue-specific δ^{15}N values of amino acids in several aquatic organisms including fish (e.g., for muscle, scale, blood, bone, etc.), gastropods (i.e., for muscle vs shell protein), and a turtle (i.e., for yolk vs embryo) to demonstrate applicability of these tissues in the CSIA-AA. The results reveal that the δ^{15}N values of amino acids in muscle are substantially identical to those in scale, fin, and yolk of fish as well as in shell of gastropods, but not to those in internal tissues (e.g., blood, embryo, etc.). Based on these results, we concluded that the former tissues (e.g., scale and shell) are applicable as alternatives to muscle whereas the latter tissues will be required further evaluation if we can use them in the CSIA-AA.

1.3 **Illustrating isoscape map of coastal areas**

We illustrated the isoscape map (i.e., spatial variation in the $\delta^{15}N_{Baseline,Phe}$) along Sagami Bay where has complex inputs of isotopically-distinct nitrogen sources derived from ocean current and human activities. The isoscape map illustrated shows that, across Sagami Bay, there are low (~2‰), middle (~12‰), and high (~16‰) values for the $\delta^{15}N_{Baseline,Phe}$, which is simply attributable to nutrients found in ocean current, released from industrial, and experienced by denitrification, respectively (Fig. 2). Moreover, the TP calculated shows that studied two fish (generalist species) have kept a constant TP at the species level in Sagami Bay, even Sagami Bay is expected to have a large heterogeneity in the biomass size and species diversity of primary producers related to multiple nitrogen sources inputs.

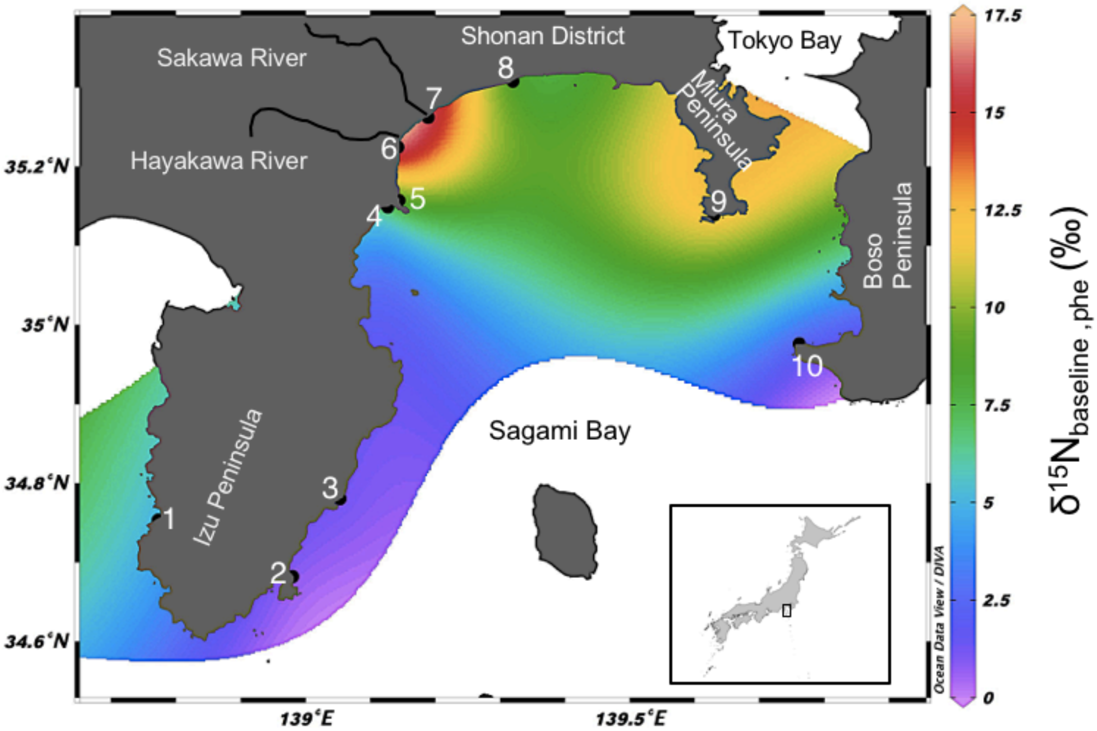

Fig. 2. $\delta^{15}N_{Baseline,Phe}$ isoscape map of Sagami Bay. Color gradient bar indicates the $\delta^{15}N_{Baseline,Phe}$ values. Number 1-10 represents the sampling sites.

Study 2: Evaluating the energy consumption of organisms in ocean acidification

The objectives of study 2 is to evaluate the energy consumption of marine species in conditions with a lower pH of seawater compared to modern ocean. This is because that CO_2 released from the industry decreases ocean pH by 0.1 during the last two century and will further by 0.2-0.3 at the end of 21 century, and that marine organisms may receive stresses and therefore change energy consumption in the life.

We found that the energy consumption of fish and gastropods is decreased significantly (e.g., by 33% for a studied fish) in an experimental condition compared to control one. However, we also found that the energy consumption of corals is changed negligibly and increased significantly in the pH condition higher and lower than 7.6, respectively.

Reference

Chikaraishi Y., Ogawa N. O., Kashiyama Y., Takano Y., Suga H. and others (2009) Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnol Oceanogr Methods* 7: 740−750.