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Trophic niche separation of two non-spinose planktonic foraminifers *Neogloboquadrina dutertrei* and *Pulleniatina obliquiloculata*

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Abstract

Based on laboratory observations, planktonic foraminifers are omnivorous, feeding zooplankton and phytoplankton. Spinose species tend toward greater dependence on zooplankton prey than on phytoplankton prey, while non-spinose species are more adapted to herbivorous diets. However, the trophic activity of planktonic foraminifers in the natural environment and their trophic positions in the marine food web have not yet been fully understood. The trophic position (TP) of two non-spinose species, *Neogloboquadrina dutertrei* and *Pulleniatina obliquiloculata*, was determined by differences in the nitrogen isotopic composition between two amino acids (glutamic acid and phenylalanine). Results show that TP values of *N. dutertrei* were ~ 2.4, indicating dependence on omnivorous (mixed herbivorous and carnivorous) diets, while those of *P. obliquiloculata* were ~ 2.1, indicating dependence on herbivorous diets. Together with previous laboratory observations, these TP values suggest that *N. dutertrei* is a detritivore or scavenger, while *P. obliquiloculata* is generally a herbivore. This trophic niche separation likely allows these two planktonic foraminiferal species to live within a similar depth zone in the open water column and provides a clue for understanding causes of spatial and temporal changes in their relative abundances in living and sediment assemblages.

Keywords: Amino acid, Herbivore, Nitrogen isotopic composition, Omnivore, Planktonic foraminifers, Trophic position

1 Introduction

Planktonic foraminiferal species and assemblages are useful biological proxies for paleo-sea surface water properties (e.g., Berger 1969; Fischer and Wefer 1999; Kucera 2007) because the distribution and abundance of planktonic foraminiferal species are mainly controlled by environmental factors such as sea surface temperature, salinity, dissolved oxygen, light attenuation, and nutrient availability (e.g., Hemleben et al. 1989; Murray 1991; Watkins et al. 1998; Kuroyanagi and Kawahata 2004; Schiebel and Hemleben 2017). Biological factors such as the abundance of prey and predators may also control the distribution and abundance of planktonic foraminifers, especially on regional and seasonal scales dominated by upwelling (Thiede 1975; Ortiz et al. 1995; Schiebel et al. 2004). Laboratory observations of trophic activity have revealed that planktonic foraminifers are basically known as omnivorous, feeding zooplankton and phytoplankton. Spinose species tend toward greater dependence on zooplankton prey than on phytoplankton prey, while non-spinose species are more adapted to herbivorous diets (e.g., Anderson et al. 1979; Spindler et al. 1984). Diet preferences might also be diverse among different genera and species to avoid diet competition in the same water column. Such trophic niche separation might partly explain the diversity and distribution of planktonic foraminifers living in the surface to subsurface water column and their speciation among different species within a genus or among different genotypes within a morphospecies

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(Weiner et al. 2012). However, the trophic activity of planktonic foraminifers in the natural environment and their trophic positions in the marine food web have not yet been fully understood.

Trophic position can be estimated based on the nitrogen isotopic composition ($\delta^{15}N$) of two amino acids (glutamic acid and phenylalanine) in consumer organisms (Chikaraishi et al. 2009). Each amino acid experiences different isotopic fractionation during amino acid metabolism. Glutamic acid shows a large $\delta^{15}N$ enrichment from one trophic level to the next, whereas phenylalanine shows little change in the $\delta^{15}N$ value between trophic levels. The use of the $\delta^{15}N$ differences between these two amino acids is a powerful tool for elucidating the trophic position of organisms in aquatic ecosystems with an error of 0.1–0.2 units (Chikaraishi et al. 2009). The traditional trophic position estimation techniques that rely on the $\delta^{15}N$ values of bulk consumer tissues are sensitive to background isotopic variation between the basal resources of a food web and are hampered by spatial and temporal variations in the $\delta^{15}N$ value of primary producers. In contrast, the trophic position based on $\delta^{15}N$ values of glutamic acid and phenylalanine from a single consumer is independent of such factors and successfully applied in studying the trophic position of aquatic organisms (Chikaraishi et al. 2014; Ohkouchi et al. 2017). Another advantage of the amino acid $\delta^{15}N$ approach is that it permits analyses of small specimens (2 nmol for each amino acid; Chikaraishi et al. 2009), which allows us to assess the trophic functions of innumerable meiofauna such as foraminifers (Tsuchiya et al. 2018).

This amino acid $\delta^{15}N$ approach differs from a recently advanced planktonic foraminiferal shell-bound $\delta^{15}N$ approach, which measures $\delta^{15}N$ in organic matter bound within the calcareous shells (e.g., Ren et al. 2009, 2012; Schiebel et al. 2018; Smart et al. 2018, 2020). Planktonic foraminifers are expected to track the $\delta^{15}N$ of the organic matter produced in the surface ocean. The $\delta^{15}N$ values of bulk tissue and shell-bound nitrogen are similar in absolute value and vary together, supporting the use of shell-bound nitrogen as a recorder of upper ocean $\delta^{15}N$ changes (Smart et al. 2018). In oligotrophic subtropical sites, shell-bound $\delta^{15}N$ values in modern surface sediments and net tows are strongly correlated with the $\delta^{15}N$ values of thermocline (i.e., shallow subsurface water) nitrate (Ren et al. 2012; Smart et al. 2018, 2020). Thus, N_2 fixation and denitrification changes are recorded in foraminiferal shell-bound $\delta^{15}N$.

In this paper, we apply the amino acid $\delta^{15}N$ approach to determine the trophic position of two non-spinose planktonic foraminiferal species, *Neogloboquadrina dutertrei* (d'Orbigny, 1839) and *Pulleniatina obliquiloculata* (Parker and Jones, 1865), both of which are common in tropical to subtropical, warm subsurface water around the deep chlorophyll maximum (DCM) and generally herbivorous (Hemleben et al. 1989; Schiebel and Hemleben 2017). Results of this study imply the trophic niche separation of coexisting planktonic foraminifers in the subsurface water column and a clue for understanding causes of spatial and temporal changes in their relative abundances in living and sediment assemblages.

2 Materials and methods

2.1 Hydrographic data and plankton tow samples

Hydrographic data and plankton tow samples were collected from 0 to 200 m in water depth during the R/V *Tansei-Maru* cruise (KT-11-25) in the afternoon on October 14, 2011 (full moon phase), at the mouth of Suruga Bay, Japan (34°38.989′N, 138°33.045′E), where the Kuroshio Current (warm current) flowed off south of Kouzushima Island to the northeast (Fig. 1). Hydrographic data including temperature, salinity, density, dissolved oxygen, and chlorophyll *a* (Chl *a*) were measured near the plankton tow sampling site by a conductivity temperature depth sensor (CTD; SBE 9, Sea-Bird Scientific) and a fluorometer (Aquatracka MkIII, Chelsea Technologies Group Ltd.). The upper 200 m was sampled twice using a Vertical Multiple Plankton Sampler (VMPS, Tsurumi-Seiki Co. Ltd.). The tow sampler has a 0.25-m^2 opening and 100-μm mesh size (NXX13). Four depth intervals (0–20, 20–50, 50–100, and 100–200 m) were sampled during each tow. The samples were fixed with seawater-buffered 5% formalin in a 50-mL vial. Formalin fixation does not affect $\delta^{15}N$ values of amino acids derived from an aquatic consumer (Ogawa et al. 2013).

Samples were poured into petri dishes. Planktonic foraminifers alive at the time of sampling, which were identified by the presence of cytoplasm, were picked from a wet solution by pipetting using a Pasteur pipette, mounted on a microfossil slide, identified, and counted to the species level. Microphotographs of five common species (*Neogloboquadrina dutertrei*, *Pulleniatina obliquiloculata*, *Globigerina bulloides* d'Orbigny, 1826, *Trilobatus sacculifer* (Brady, 1877), *Globigerinoides ruber* white (d'Orbigny, 1839)) were taken using a digital camera attached to a binocular dissecting microscope. The maximum diameter was measured using Image J (NIH) to estimate the size-frequency distribution of each species at each depth interval (Additional file 1: Fig. S1). Since other zooplankton were more abundant than planktonic foraminifers, the remaining samples were divided into 2–5 mL subsamples by pipetting from stirred and homogenized samples according to methods described in Omori and Ikeda (1976). Zooplankton alive at the time of sampling, which were identified by the presence of organic matter, were picked from a wet solution by pipetting using a Pasteur

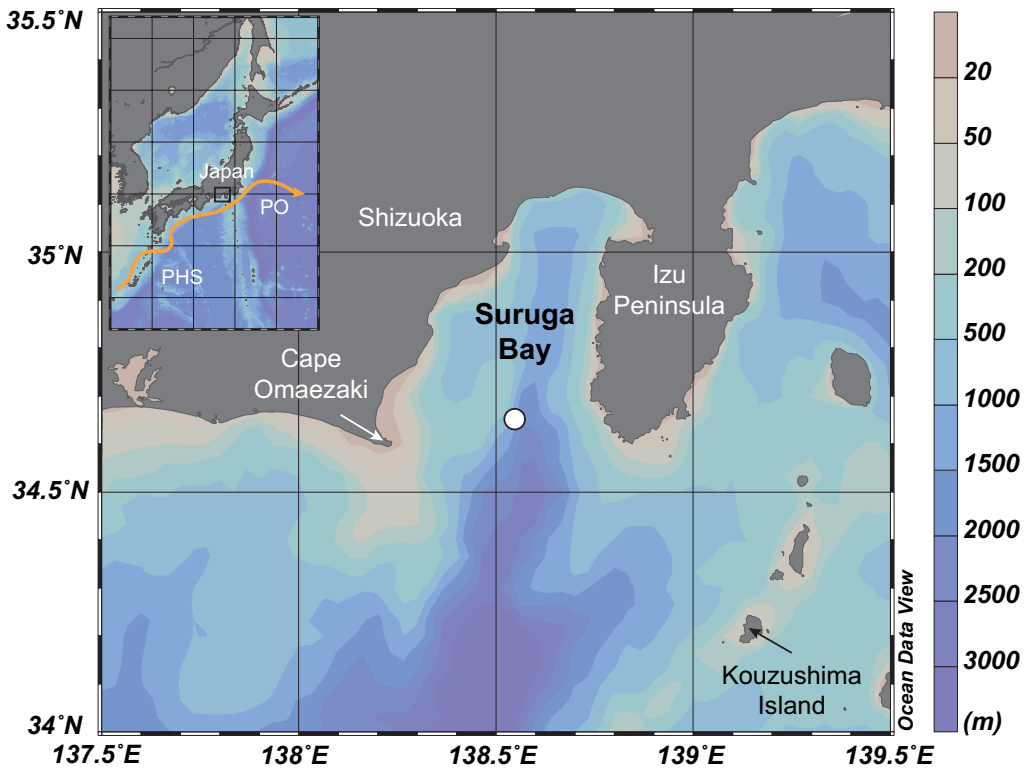

Fig. 1 Location of a plankton tow site (white circle) in Suruga Bay, Japan. Orange line in the insert map indicates the Kuroshio current at the time of sampling. *PO* Pacific Ocean, *PHS* Philippine Sea

pipette and stored in a glass vial filled with seawater-buffered 5% formalin. The zooplankton were identified and counted (Suidosha, Co., Ltd.) and finally classified to the order level. Counts of planktonic foraminifers and zooplankton were converted to the standing stock (SS; the number of individuals m^{-3} seawater) using the following equations:

$$\mathrm{SS}_{\text{planktonic foraminifers}} = n_{\text{sample}} \div v_{\text{seawater}} \quad (1)$$

$$\mathrm{SS}_{\text{zooplankton}} = n_{\text{subsample}} \div v_{\text{subsample}} \times v_{\text{sample}} \div v_{\text{seawater}} \quad (2)$$

where n is the count in a sample or subsample (individuals), $v_{\text{subsample}}$ is the volume of a subsample (2–5 mL), v_{sample} is the volume of a sample (50 mL), and v_{seawater} is the volume of seawater. Since the volume of seawater filtered by a plankton net was not obtained using a flow meter, v_{seawater} is calculated by the opening area of a plankton sampler (0.25 m^2) multiplied by the water depth interval (20–100 m).

In order to estimate the abundance of particulate organic matter (POM), the remaining residue excluding planktonic foraminifers and zooplankton (referred to as residue POM) and another 5-mL subsample (referred to as total POM) were filtered, wet weighed, and converted to the wet weight of POM (mg m^{-3}) using the following equation:

$$\mathrm{POM} = w_{\text{subsample}} \div v_{\text{subsample}} \times v_{\text{sample}} \div v_{\text{seawater}} \quad (3)$$

where $w_{\text{subsample}}$ is the mass of POM in a subsample.

In order to correlate the standing stock data with environmental variables, the median of environmental variables such as temperature, salinity, density, dissolved oxygen, and Chl *a*, was calculated for each depth interval. Standing stock data of planktonic foraminifers (*N. dutertrei*, *P. obliquiloculata*) and zooplankton (Calanoida, Cyclopoida, Poecilostomatoida, Harpacticoida, all copepods, other zooplankton, all zooplankton) and the abundance of POM (residue and total) were square-root transformed. Transformed standing stock data of planktonic foraminifers were correlated with other variables (environmental, zooplankton, POM) using Pearson's correlations. In addition, the standing stock data were analyzed with a linear model using Chl *a* and total POM as explanatory variables to examine the effects of biological variables. These statistical analyses were conducted in R 4.1.1 (R Core Team 2021). We also

calculated correlations between the standing stock data and the mean of environmental variables. The correlation results using the mean were similar to those using the median.

2.2 Amino acid nitrogen isotope analysis

In this study, we measured $\delta^{15}N$ values of the bulk cells (i.e., the sum of the cell cytoplasm, organic membranes, and intracrystalline protein in a shell) of fixed specimens of *N. dutertrei* and *P. obliquiloculata*. Since the amount of intracrystalline proteins is fewer than the cell cytoplasm, $\delta^{15}N$ values in the bulk cells would be expected to indicate the short-term value of their metabolism. Due to the low number of specimens, fixed specimens from two replicate samples for each water depth interval were pooled for the bulk cell analysis (i.e., one pooled sample of each water depth interval was measured for each species). The number of *N. dutertrei* and *P. obliquiloculata* specimens measured for each water depth interval ranged from 18 to 353 and 14 to 137, respectively. Measured test size ranged from 150 to 650 μm for all depth intervals of both species.

The $\delta^{15}N$ value of amino acids was determined according to Chikaraishi et al. (2009). Briefly, each specimen was hydrolyzed in 12 M HCl at 110 °C, and then, the hydrolyzate was washed with *n*-hexane/dichloromethane (3:2, v/v) to remove any hydrophobic constituents. After derivatization with thionyl chloride/2-propanol (1:4, v/v) and subsequently with pivaloyl chloride/dichloromethane (1:4, v/v), the derivatives of the amino acids were extracted with *n*-hexane/dichloromethane (3:2, v/v). The $\delta^{15}N$ value of individual amino acids was determined by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) using a Delta^{plus}XP isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled with a 6890 N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) via combustion and reduction furnaces. $\delta^{15}N$ value is defined as $\delta^{15}N = ([^{15}N/^{14}N]_{sample}/[^{15}N/^{14}N]_{Air} - 1) \times 1000$ and expressed in conventional (‰) against that of air (Air).

The trophic position ($TP_{Glu/Phe}$) of the sample was calculated based on the following equation proposed by Chikaraishi et al. (2009):

$$TP_{Glu/Phe} = \left(\left(\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4\right)/7.6\right) + 1 \quad (4)$$

where $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ represent the $\delta^{15}N$ values of glutamic acid and phenylalanine, respectively, 3.4 is the isotopic difference between glutamic acid and phenylalanine in primary producers, and 7.6 is the offset of the trophic discrimination factor of these two amino acids per trophic position increase. Although this equation may need modification (particularly for the trophic discrimination factor) for some specific organisms, such modification is not required for organisms in the lower-trophic-level hierarchy of food webs (McMahon and McCarthy 2016). The trophic position is expected to be 1.0 for a "pure" primary producer and 2.0 for a "pure" primary consumer.

Propagation error (potential uncertainty) in $TP_{Glu/Phe}$ value has been calculated by taking into account the propagation of 1σ for $\delta^{15}N_{Glu}$, $\delta^{15}N_{Phe}$, the isotopic difference between glutamic acid and phenylalanine in primary producers, and the offset of the trophic discrimination factor (TDF) of these two amino acids per trophic position increase in Eq. (4) (Chikaraishi et al. 2009, 2014), and is expressed in the following equation (Kruse et al. 2015):

$$1\sigma_{TL} = \left[\left(y^2/7.6x\right) + (1\sigma_{TDF}/TDF)^2\right]^{1/2} \quad (5)$$

where $x = \delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4$, $y = [2(1\sigma_m)^2 + (1\sigma_\beta)^2]^{1/2}$, $1\sigma_m = 0.5‰$, $1\sigma_\beta = 0.9‰$, and $1\sigma_{TDF} = 1.2‰$, after Chikaraishi et al. (2009). Previous studies have indicated that the potential uncertainty in the $TP_{Glu/Phe}$ value calculated via propagation of error is 0.2–0.4 for each trophic level based on an assumed standard deviation of 0.5‰ (1σ) for the observed $\delta^{15}N$ values of glutamic acid and phenylalanine (Chikaraishi et al. 2009).

3 Results

3.1 Environmental variables

Water temperature was relatively stable (approx. 22.7 °C) at 0–40 m depth, and the thermocline was located at 40–60 m depth (Fig. 2, Additional file 2: Table S1). Salinity was stable (approx. 34.0) at 0–40 m depth, and the pycnocline was located at 40–60 m depth (Fig. 2). Chl *a* concentrations were 0.3 μM on average at 0–49 m depth, showed the highest peak (1.1 μM) at 50 m depth, with the deep chlorophyll maximum (DCM) being located in the center of the pycnocline, and then gradually decreased down to 0.04 μM at a deeper depth. Dissolved oxygen was stable (4.7 ml L^{-1} on average) at 0–40 m depth and then decreased at 40–60 m across the pycnocline.

3.2 Planktonic foraminifers

A total of 11 planktonic foraminiferal species were identified (Fig. 3, Additional file 2: Table S2). The assemblage was mainly composed of *N. dutertrei*, *P. obliquiloculata*, *G. bulloides*, *T. sacculifer*, and *G. ruber* white. Of these five species, *N. dutertrei*, *P. obliquiloculata*, and *G. bulloides* were common at all the water depths sampled and account for > 90% of the total foraminiferal standing

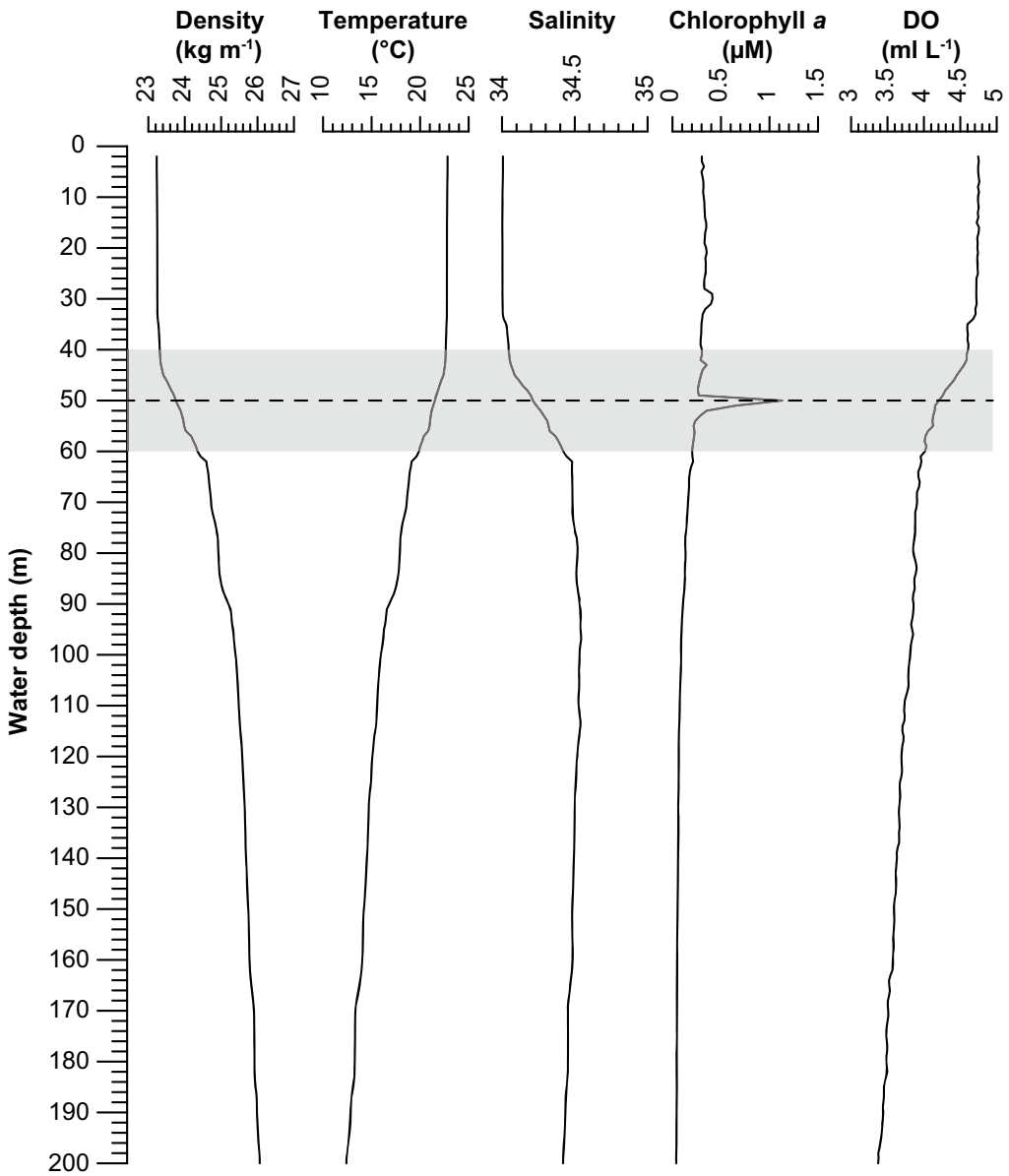

Fig. 2 Hydrographic conditions at the plankton tow site in Suruga Bay, Japan. A gray zone in a indicates the thermocline/halocline/pycnocline, while a dashed line indicates the deep chlorophyll maximum (DCM)

stock. The standing stock of planktonic foraminifers was high in the upper 50 m. The highest value (~ 50 individuals m^{-3}) was recovered in the upper 20 m. *N. dutertrei* and *P. obliquiloculata* were more common in the upper 50 m but decreased at water depth below 50 m. The standing stock of *G. bulloides* was reasonably uniform (2.8 individuals m^{-3}) throughout the water depths sampled. *T. sacculifer* and *G. ruber* white were low in abundances (~ 1 individual m^{-3}) throughout the water depths sampled.

3.3 Zooplankton and particulate organic matters

Zooplankton, excluding planktonic foraminifers, were abundant in the upper 50 m, gradually decreasing at deeper depth (Fig. 4, Additional file 2: Table S3). Copepods were the most common zooplankton, accounting for more than 80% of the total zooplankton standing stock. Of the copepod assemblage, Calanoida, Cyclopoida, and Poecilostomatoida were more common than other taxa, comprising more than 95% of the assemblage. Decreasing zooplankton abundance

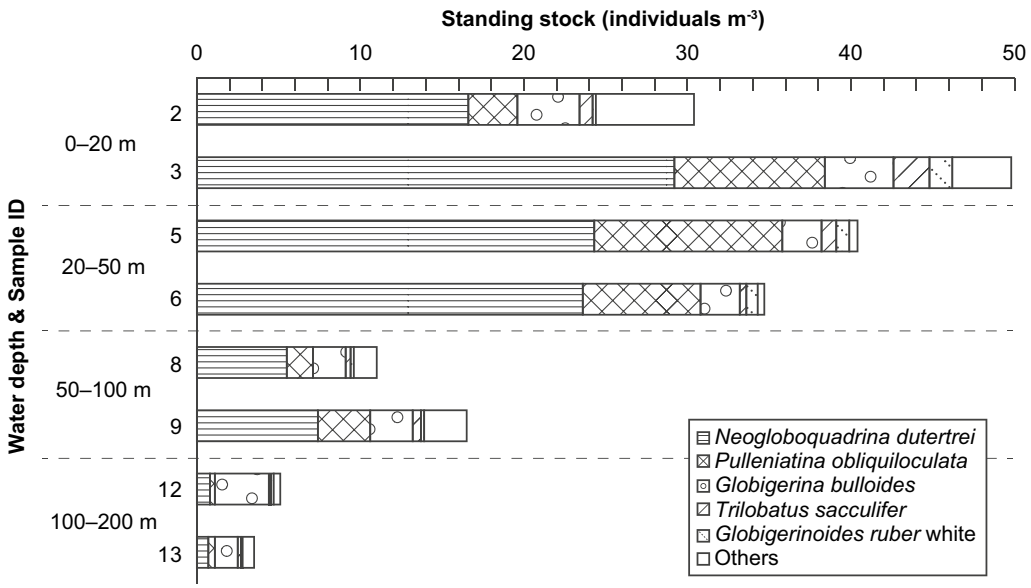

Fig. 3 Standing stocks of living planktonic foraminifers at four depth intervals (0–20, 20–50, 50–100, and 100–200 m; two replicates) in Suruga Bay, Japan

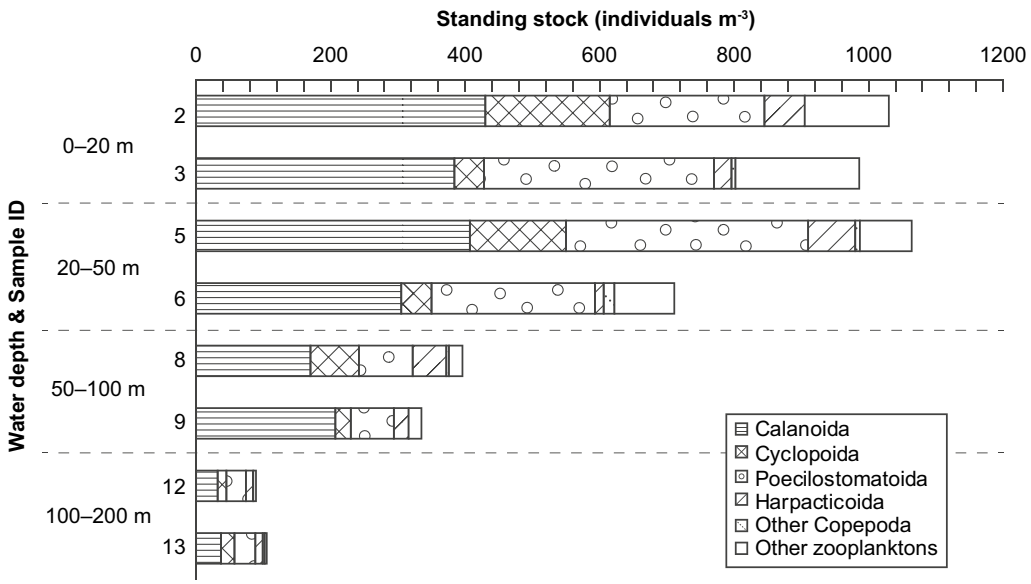

Fig. 4 Standing stocks of living zooplankton at four depth intervals (0–20, 20–50, 50–100, and 100–200 m; two replicates) in Suruga Bay, Japan

with depth was mostly explained by the standing stocks of Calanoida and Poecilostomatoida, both of which showed the maximum abundance of 382 and 294 individuals m^{-3} in the upper 50 m, respectively. Total POMs were also abundant in the upper 50 m (~ 213 mg m^{-3}), decreasing at deeper depth (Additional file 2: Table S3).

3.4 Correlations and regression with biological variables

The standing stock of *N. dutertrei* was highly correlated with most environmental and biological variables (> 0.9; Additional file 2: Table S4). The standing stock was highly correlated with temperature (0.98), salinity (− 0.91), and Chl *a* (0.97), while it was weakly correlated with standing stocks of Cyclopoida (0.57) and Harpacticoida (0.46). The standing stock of *P. obliquiloculata* was highly correlated with most environmental and biological variables (> 0.8). In particular, the standing stock was highly correlated with that of Poecilostomatoida (0.93), temperature (0.88), salinity (− 0.81), and Chl *a* (0.86), while it was weakly correlated with residue POM (0.57), standing stocks of Cyclopoida (0.46) and Harpacticoida (0.43).

Linear models indicate that standing stocks of both species were significantly affected by Chl *a* (*N. dutertrei*, $p < 0.0001$; *P. obliquiloculata* $p < 0.05$), but not by total POM (*ns*, Table 1). Both species were highly correlated with Chl *a* concentration distribution over the upper water column (Fig. 5). Linear regression analysis indicates that the standing stock of *N. dutertrei* was explained as $N_{N.\ dutertrei} = 13.65$ Chl $a + 0.53$ ($R^2 = 0.95$, adjusted $R^2 = 0.94$, $p < 0.00005$), while that of *P. obliquiloculata* was explained as $N_{P.\ obliquiloculata} = 6.75$ Chl $a + 0.64$ ($R^2 = 0.74$, adjusted $R^2 = 0.70$, $p < 0.006$).

3.5 Nitrogen isotopic composition of amino acids and estimated $TP_{Glu/Phe}$

Observed $TP_{Glu/Phe}$ values are averages based on bulk specimens of each foraminiferal species and characterized by a low propagation error (potential uncertainly) (Table 2, Additional file 2: Table S5). However, since the propagation error was significant (0.67) for *P. obliquiloculata* at 100–200 m depth, the value was not used for further discussion. $TP_{Glu/Phe}$ values for bulk specimens of both species were similar through the water column (Fig. 6). The $TP_{Glu/Phe}$ values for bulk specimens of *P. obliquiloculata* (~ 2.1) were lower than the values for bulk specimens of *N. dutertrei* (~ 2.4). The exception was $TP_{Glu/Phe}$ values at 20–50 m depth, similar for both species (~ 2.3).

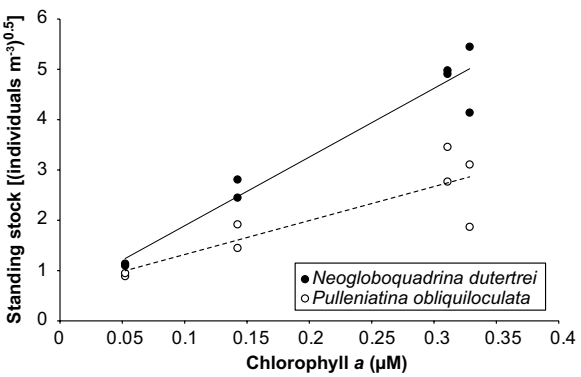

Fig. 5 Correlations of standing stocks of two non-spinose planktonic foraminiferal species (*Neogloboquadrina dutertrei* and *Pulleniatina obliquiloculata*) with chlorophyll *a* concentration

4 Discussion

The water column in the study area consisted of three layers including (1) the surface mixed layer, (2) the thermocline/halocline/pycnocline at the 40–60 m depth interval comprising the DCM, and (3) the subsurface layer below 60 m depth. These observations are consistent with previously reported water mass structure in Suruga Bay, where mixed coastal and outer surface water (0–50 m depth) covers outer Kuroshio water characterized by high salinity (100–200 m depth) (Nakamura and Muranaka 1979). The surface mixed layer and the thermocline/halocline/pycnocline are warmer and more productive than the subsurface water column and accommodate the highest standing stocks of planktonic foraminifers and zooplankton, as shown in high correlations with environmental variables. However, it is difficult to determine their primary limiting factors because many environmental variables covary with water depth.

N. dutertrei and *P. obliquiloculata* were dominant species in the planktonic foraminiferal assemblage of the upper 50 m. Both species generally show the maximum abundance around the DCM (Schiebel and Hemleben 2017). *N. dutertrei* is generally distributed in tropical to temperate open oceans and upwelling settings, showing

Table 1 Results of the linear model for standing stocks of *Neogloboquadrina dutertrei* and *Pulleniatina obliquiloculata*

Species	Variable	DF	SS	MS	*F*	*p*
Neogloboquadrina dutertrei	Chl *a*	1	20.0022	20.0022	88.4992	0.00023
	Total POM	1	0.0365	0.0365	0.1616	0.70433
	Residuals	5	1.1301	0.226		
Pulleniatina obliquiloculata	Chl *a*	1	4.8938	4.8938	15.0575	0.01164
	Total POM	1	0.0762	0.0762	0.2344	0.64875
	Residuals	5	1.625	0.325		

DF, degrees of freedom; SS, Sum of squares; MS, mean squares; *F*, *F* ratio; *p*, probability value

Table 2 Nitrogen isotopic composition of glutamic acid ($\delta^{15}N_{Glu}$) and phenylalanine ($\delta^{15}N_{Phe}$), and estimated trophic position ($TP_{Glu/Phe}$) and the propagation error of two non-spinose planktonic foraminiferal species (*Neogloboquadrina dutertrei* and *Pulleniatina obliquiloculata*)

Species	Water depth (m)	Number of specimens	$\delta^{15}N_{Glu}$ (‰, relative to air)	$\delta^{15}N_{Phe}$ (‰, relative to air)	$TP_{Glu/Phe}$	Propagation error
Neogloboquadrina dutertrei	0–20	202	15.8	1.5	2.44	0.20
	20–50	353	13.6	0.0	2.34	0.20
	50–100	50	13.3	0.3	2.26	0.20
	100–200	18	15.9	1.0	2.52	0.20
Pulleniatina obliquiloculata	0–20	60	11.4	-0.3	2.09	0.21
	20–50	137	14.1	0.9	2.28	0.20
	50–100	45	10.1	0.3	1.84	0.23
	100–200	14	7.1	3.3	1.05	0.67

Propagation error in $TP_{Glu/Phe}$ was calculated in Eq. (5)

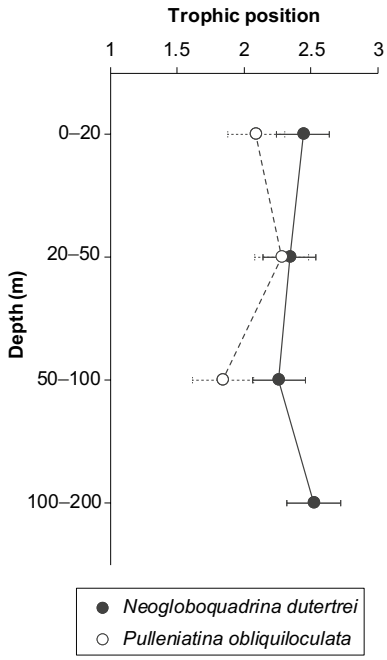

Fig. 6 Trophic position ($TP_{Glu/phe}$) of two non-spinose planktonic foraminiferal species (*Neogloboquadrina dutertrei* and *Pulleniatina obliquiloculata*) along four depth intervals in Suruga Bay, Japan. Error bars indicate the propagation error (potential uncertainly) in $TP_{Glu/Phe}$ calculated in Eq. (5)

the maximum standing stocks at the DCM (e.g., Ravelo et al. 1990; Schmuker and Schiebel 2002; Watkins et al. 1996, 1998). In water columns around the Japanese Islands, *N. dutertrei* is found below the pycnocline, and its abundance peaks just below the DCM (Kuroyanagi and Kawahata 2004). *P. obliquiloculata* is generally distributed in tropical to subtropical surface mixed layer around the thermocline and DCM (e.g., Watkins et al. 1996, 1998; Jentzen et al. 2018). These observations are consistent with high positive correlations of the standing stocks of both species with Chl *a* in this study and in previous studies (Watkins et al. 1998), although no correlation was observed for *N. dutertrei* in similar settings (Kuroyanagi and Kawahata 2004).

The $TP_{Glu/Phe}$ value of a predator is one level higher than that of its prey. The $TP_{Glu/Phe}$ values of *N. dutertrei* are ~ 2.4, indicating an omnivorous feeding strategy, while those of *P. obliquiloculata* are ~ 2.1, indicating dependence mostly on herbivorous diets. Similar $TP_{Glu/Phe}$ values at 20–50 m depth indicate that *P. obliquiloculata* becomes more omnivorous near the DCM than above. Previous studies using a variety of marine and terrestrial organisms demonstrated that even if the $\delta^{15}N$ value of amino acids has a variation between individuals of a species, their trophic position can remain unchanged during a given period, even though its food type and/or source has changed dramatically (Chikaraishi et al. 2014).

Laboratory studies demonstrated that diatoms are a significant part of many non-spinose species' diets and are found in digestive vacuoles in these two species (Anderson et al. 1979; Spindler et al. 1984). Metazoan tissues are also found in the digestive vacuoles of non-spinose species collected in the open ocean, although they can only feebly catch and hold zooplankton prey when grown in the laboratory since the rhizopodial net of non-spinose species is not suited to capture living prey like copepods (Spindler et al. 1984; Hemleben et al. 1989). Therefore, the metazoan tissue is likely obtained from inactive (e.g., dead) organisms caught in the rhizopodia or by snaring fecal matter containing incompletely digested metazoan tissue (Hemleben et al. 1989). These previous laboratory observations and $TP_{Glu/Phe}$ values in this study suggest that *N. dutertrei* is a detritivore or scavenger, while *P. obliquiloculata* generally depends on herbivorous diets.

This assumption is supported by variable Ba/Ca signals in shells of *N. dutertrei*, which are likely due to calcification in a microenvironment enriched in Ba such as marine snow or other organic particulates (Fehrenbacher et al. 2018). Although the standing stock of *N. dutertrei* is more positively correlated with Chl *a* than that of *P. obliquiloculata* (Fig. 5), this might be explained by the increasing abundance of dead metazoan tissues and fecal matters in the upper water column. Slight trophic niche separation inferred from this study may allow these two planktonic foraminiferal species to live within a similar depth zone in the open water column.

Some non-spinose species, including *N. dutertrei* and *P. obliquiloculata*, appear to facultatively harbor algal endobionts, chrysophycophytes (chrysophytes), which are capable of photosynthesizing within the perialgal vacuoles (Gastrich 1987; Takagi et al. 2019). The algae are also frequently observed in stages of division within the host vacuoles, thus indicating they are in a healthy state (Hemleben et al. 1989). However, some specimens are entirely devoid of these algae, and some algae were observed in a state of being digested by the foraminifers (Hemleben et al. 1989). Predation on algal endobionts results in $TP_{Glu/Phe}$ value around two due to a predator–prey interaction in terms of the amino acid metabolism between host and endobiont, while the use of photosynthate from algal endobionts as a nitrogen source results in $TP_{Glu/Phe}$ value around one (Tsuchiya et al. 2018). Algal symbiont-bearing planktonic foraminifers likely obtain carbon and nitrogen from two distinct sources, the diet and the symbionts (Uhle et al. 1997). For example, the carbon and nitrogen isotopic data suggest that symbiont-bearing spinose species *Orbulina universa* is indicative of the transfer of isotopically heavy metabolic carbon and nitrogen from its symbionts and relatively lighter carbon and nitrogen from the diet. In contrast, diet is the sole source of metabolic carbon and nitrogen used for amino acid synthesis in symbiont-barren spinose species (*Globigerina bulloides*) (Uhle et al. 1997). Intermediate depth-dwelling and chrysophyte endobiont-bearing *N. dutertrei* and *P. obliquiloculata* exhibit intermediate ranges of tissue and shell-bound $\delta^{15}N$ values between the low-$\delta^{15}N$ surface-dwelling and dinoflagellate symbiont-bearing and high-$\delta^{15}N$ subsurface-dwelling, symbiont-barren species, but more similar to the latter group (Smart et al. 2018, 2020). These previous studies and our results on $TP_{Glu/Phe}$ values suggest that these two non-spinose species do not have a symbiotic relationship with algal endobionts, but hold algal endobionts to prey on them. Further studies are necessary to reveal a relative dependence between foods and algal endobionts for nitrogen sources of these non-spinose species.

Recently advanced analytical techniques of $\delta^{15}N$ values of shell-bound organic matter of planktonic foraminifera (shell-bound $\delta^{15}N$) are potential tools for reconstructing past changes in global nitrogen cycling (e.g., Ren et al. 2009, 2012; Schiebel et al. 2018). Late Quaternary glacial-interglacial records indicate that shell-bound $\delta^{15}N$ was higher during the glacial periods than the interglacial periods, suggesting that sea-level driven oscillations in the balance of N_2 fixation and denitrification (e.g., Ren et al. 2009, 2017). Future studies of shell-bound amino acid $\delta^{15}N$ approaches combined with shell-bound organic matter $\delta^{15}N$ analyses would provide information on temporal changes in paleo-trophic levels and food webs associated with glacial-interglacial changes in ocean productivity.

Trophic niche separation of *N. dutertrei* and *P. obliquiloculata* revealed in this study may also provide a clue for understanding the causes of spatial and temporal changes in their relative abundances in sediment assemblages. For example, in the East China Sea and the Ryukyu Arc regions, both species are common under the influence of Kuroshio Current, based on surface sediments (Ujiié et al. 2003) and sediment trap data (Xu et al. 2005). In contrast, temporal variations of the relative abundances of *N. dutertrei* and *P. obliquiloculata* from the last glacial to Holocene at the Okinawa Trough show a negative relationship (Li et al. 1997; Ujiié and Ujiié 1999; Ujiié et al. 2003). *N. dutertrei* was common in the last glacial period and the Holocene *Pulleniatina* Minimum Event (PME; ca. 4.5–3 ka), while *P. obliquiloculata* increased toward the interglacial but decreased during the PME (Li et al. 1997; Ujiié and Ujiié 1999; Ujiié et al. 2003; Lin et al. 2006). In lower latitudes of the West Pacific, the negative relationship of both species is less obvious (Lin et al. 2006). Temporal changes in ocean productivity associated with these paleoceanographic changes may have influenced food webs and these two species with slightly different trophic levels.

5 Conclusions

The trophic position ($TP_{Glu/Phe}$) of two non-spinose species, *N. dutertrei* and *P. obliquiloculata*, was determined by differences in the nitrogen isotopic composition between two amino acids (glutamic acid and phenylalanine). The $TP_{Glu/Phe}$ values of *N. dutertrei* were ~ 2.4, indicating an omnivorous feeding strategy, while those of *P. obliquiloculata* were ~ 2.1, indicating dependence mostly on herbivorous diets. The $TP_{Glu/Phe}$ values in this study, together with previous laboratory observations, suggest that *N. dutertrei* is a detritivore or scavenger, while *P. obliquiloculata* is generally a herbivore. The $TP_{Glu/Phe}$ values also suggest that these two non-spinose species do not have a symbiotic

relationship with algal endobionts, but hold those algal endobionts to prey on them. This trophic niche separation may allow these two planktonic foraminiferal species to live within a similar depth zone in the open water column and provide a clue for understanding causes of spatial and temporal changes in their relative abundances in living and sediment assemblages.

Abbreviations

Chl *a*: Chlorophyll *a*; DCM: Deep chlorophyll maximum; Glu: Glutamic acid; Phe: Phenylalanine; PME: Holocene *Pulleniatina* minimum event; POM: Particulate organic matter; TP: Trophic position.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40645-022-00478-3.

Additional file 1: Fig. S1. Size-frequency distribution of two non-spinose planktonic foraminiferal species (*Neogloboquadrina dutertrei* and *Pulleniatina obliquiloculata*) collected at four depth intervals (0–20, 20–50, 50–100, and 100–200 m; two replicates) in Suruga Bay, Japan.

Additional file 2. **Table S1**. Hydrographic data in the upper 200 m at the plankton tow site (Suruga Bay, Japan). **Table S2**. Standing stock data (individuals m^{-3}) of living planktonic foraminifers at four depth intervals (0–20, 20–50, 50–100, and 100–200 m; two replicates) in Suruga Bay, Japan. **Table S3**. Standing stock data (individuals m^{-3}) of living zooplankton and wet weight of particulate organic matters (POM) at four depth intervals (0–20, 20–50, 50–100, and 100–200 m; two replicates) in Suruga Bay, Japan. **Table S4**. Correlation results of two non-spinose planktonic foraminiferal species (*Neogloboquadrina dutertrei* and *Pulleniatina obliquiloculata*) with environmental and biological variables. **Table S5**. Nitrogen isotopic composition of amino acids in two non-spinose species of planktonic foraminifers. Abbreviations: alanine (Ala), glycine (Gly), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), aspartic acid (Asp), threonine (Thr), serine (Ser), methionine (Met), glutamic acid (Glu), phenylalanine (Phe), and Hydroxyproline (Hyp).

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Authors' contributions

KF and MT proposed the topic, conceived, and designed the study. MT carried out the field sampling. RT and KF carried out the plankton analysis. MT, YC, YS, and NO carried out amino acid nitrogen isotope measurements. All authors analyzed the data and helped in their interpretation. KF collaborated with the RT, MT, YC, and NO in the construction of manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Declarations

Competing interests

The authors declare that they have no competing interest.

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