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Response of planktic foraminiferal shells to ocean acidification and global warming assessed using micro-X-ray computed tomography

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Abstract. Ocean acidification is now progressing, primarily due to the fact that the oceans have absorbed about 50% of the anthropogenic CO₂ emitted since the industrial revolution. Many marine calcifying organisms, such as foraminifers and coccoliths, are known to build their shells using carbonate ions present in the seawaters surrounding them. Carbonate saturation state has a crucial influence on foraminiferal calcification, and foraminiferal shell production is known to be sensitive to increase in ocean pCO_2 . Moreover, ocean warming is also progressing along with acidification. Therefore, both environmental changes could affect foraminiferal shell formation. However, the relationship between foraminiferal shell parameters (i.e., size, weight, volume, and density) and ocean pCO_2 or sea surface temperature (SST), or both, remains unclear. In this study, we used fossil planktic foraminifer Globigerinoides ruber (white) in a late Quaternary sediment core (MD98-2196) from the East China Sea to investigate a relationship between the shell parameters and oceanographic properties estimated based on the proxies from the same core. The foraminiferal shells were scanned using high-resolution micro-X-ray computed tomography (MXCT) to determine shell volume and density. The results showed that the size-normalized weight and the size-normalized volume of the shell had a negative correlation with the SST and atmospheric pCO_2 . The negative correlation between weight/volume and atmospheric pCO_2 agrees with the previous laboratory experiments and geological record during the Pliocene. However, the correlation between weight/volume and SST should be interpreted with caution because it might be an artifact due to the correlation between SST and atmospheric pCO2. On the other hand, shell density is only weakly or insignificantly correlated with SST and pCO₂, suggesting that these environmental parameters do not exert any impact on shell density. Thus, future ocean acidification will negatively affect the carbonate productivity of planktic foraminifers, even if it will not affect shell density. The temperature effect on the shell formation of the planktic foraminifers might be much less than ocean acidification considering controversial results of the temperature sensitivity in previous studies.

Keywords: atmospheric pCO₂, Globigerinoides ruber, shell density, shell volume, size-normalized weight, SST

Introduction

Since the industrial revolution, oceans have absorbed approximately 50% of anthropogenic CO₂, thus lowering the pH of surface seawaters. This is known as "ocean acidification" (e.g. Kawahata *et al.*, 2019). The process of ocean acidification has been shown from various simulations, hydrographic surveys, and time-series data (Caldeira and Wickett, 2003; Key *et al.*, 2004; Raven *et al.*, 2005; Kump *et al.*, 2009). Although absorption by seawater mitigates the increase in atmospheric pCO_2 , oceanic uptake has reduced the surface seawater $[CO_3^{2^-}]$, and consequently, decreasing the calcite and aragonite saturation states (Orr *et al.*, 2005; Kawahata *et al.*, 2019).

Some marine organisms such as, coccolithophorids, and foraminifers build up their shells using carbonate ions in ambient seawaters. Evidence has shown that the carbonate saturation state is correlated with the rate of marine calcium carbonate production (Kleypas *et al.*, 1999) and is sensitive to elevated pCO_2 (Kleypas *et al.*,



Figure 1. The core location of MD98-2196.

1999; Riebesell *et al.*, 2000; Barker and Elderfield, 2002; Orr *et al.*, 2005; Raven *et al.*, 2005; Ridgwell and Zeebe, 2005; Moy *et al.*, 2009). Foraminifers are among the most abundant protists in the world's ocean, and their shells comprise a significant portion of inorganic carbon deposits on the seafloor (Langer, 2008); specifically, they constitute 32–80% of the total deep-marine calcite budget in the global carbonate cycle (Schiebel, 2002).

Consequently, it is critical to understand the effect of ocean acidification on the calcification of planktic foraminifers. Ever since Barker and Elderfield (2002) showed that ambient [CO3²⁻] and planktic foraminiferal sizenormalized weight (SNW) are positively correlated with high confidence, SNW has been used as a proxy for calcification of foraminiferal shells. Thereafter, the SNW of foraminifers was used to reflect the surface carbonate system (Bijma et al., 2002; Mekik and Raterink, 2008; Moy et al., 2009; Naik et al., 2010; Marshall et al., 2013, 2015; An et al., 2015; Osborne et al., 2016). On the other hand, on the assumption that the surface carbonate system has little effect on foraminiferal calcification, foraminiferal shell weight was used as a tracer for carbonate saturation state of bottom waters based on differences in dissolution after deposition (Lohmann, 1995; Broecker and Clark, 2001). The reliability of SNW as a proxy for surface seawater [CO3²⁻] is often obscure, thus it is crucial to properly understand the relationship between foraminiferal calcification and carbonate chemistry. The SNW of foraminiferal shells has been ambiguously attributed to the density or thickness of the shell wall, or both (de Villiers, 2004). However, it is not possible to give a precise value of the shell wall thickness from cross-sectional images because the shell wall thickness varies from chamber to chamber (de Villiers, 2004).

To date, it has been difficult to directly measure or calculate foraminiferal shell volume accurately due to the tiny size. For marine sediment samples, it is paramount to evaluate post-depositional alteration such as dissolution and secondary calcite precipitation of foraminiferal shells because the application of SNW may be confusing (Marshall et al., 2013; Osborne et al., 2016). Recent advances in micro-X-ray computed tomography (MXCT) have provided a novel approach to observe foraminiferal shells (Görög et al., 2012; Briguglio et al., 2016; Kinoshita et al., 2017), and MXCT investigations complete the role in estimating the post-depositional alteration (Johnstone et al., 2010; Iwasaki et al., 2015; Kontakiotis et al., 2017). Furthermore, because of their µm-level highresolution and three-dimensional (3D) scanning capacity, MXCT measurements of foraminiferal shells enable us



Figure 2. Reconstructed environmental parameters; SST (A), pCO_2 (B) and pH (C) according to age. The reconstructed atmospheric SST (Ujiié *et al.*, 2016) and pCO_2 (Petit *et al.*, 1999) are shown. pH was calculated by assuming that the total alkalinity was constant during the last 100 kyr.

to obtain datasets of shell volume, density, surface area, and diameter of foraminiferal individuals (Speijer *et al.*, 2008; Briguglio and Hohenegger, 2014; Iwasaki *et al.*, 2018; Eder *et al.*, 2019; Hohenegger *et al.*, 2019; Ujiié *et al.*, 2019).

In this study, we measured and calculated shell volumes and densities of *Globigerinoides ruber* (white), which is a typical tropical species of planktic foraminifers (e.g. Bé, 1977; Hemleben *et al.*, 1989; Schiebel and Hemleben, 2017) using MXCT, and we compared these parameters and SNW with the previously reconstructed sea surface temperature (SST) and atmospheric pCO_2 for investigating response of *G. ruber* (w) shells to ocean environmental change during the last 100 kyr. Additionally, we examined the controlling factor (shell density or shell wall thickness or both) of changing of the sizenormalized weights.

	1	_
Age (kyr)	Atmospheric pCO_2 (ppmv) ¹	Mg/Ca-derived SST (°C) ²
3.1	277.3	26.4
10.1	261.7	24.3
20.0	189.2	20.3
30.8	203.3	20.6
39.8	209.1	22.5
49.9	209.1	21.8
60.2	204.3	21.8
70.3	213.7	23.2
79.7	220.1	24.4
87.7	216.0	23.1
102.4	233.5	24.7

¹ Petit et al., 1999; ² Ujiié et al., 2016

Material and methods

Sample selection

The piston core MD98-2196 used in this study was collected during the IMAGES-IV cruise in 1998. This core was drilled in the Okinawa Trough, East China Sea $(29^{\circ}52.58'N, 128^{\circ}36.50'E)$ (Figure 1), with a water depth of 951 m. This water depth is much shallower than the depth of the modern lysocline (~1,600 m) in the East China Sea (Horikawa *et al.*, 2015). Thus, expectedly, foraminiferal shells were not severely dissolved.

The core has a total length of 3,888 cm and generally comprises homogeneous clay to silty clay, interrupted by 12 tephra layers, and four fine silt layers (Ujiié and Ujiié, 2006; Ujiié et al., 2016). The chronology was obtained based on a graphic correlation between the reference curve LR04 (Lisiecki and Raymo, 2005) and stable oxygen isotope ratio (δ^{18} O) of the planktic foraminifer *Glo*bigerinoides sacculifer (Ujiié and Ujiié, 2006; Ujiié et al., 2016) before 50 ka and accelerator mass spectrometer (AMS) ¹⁴C ages for the last 50 kyr (Ujiié et al., 2016). We used an age model that had been established by Ujiié et al. (2016). AMS ¹⁴C age analysis was performed on planktic foraminiferal shells of Neogloboquadrina dutertrei from samples at 0 cm and 92 cm. Also, MARINE13 calibration curve in the CALIB 7 radiocarbon calibration program (Reimer *et al.*, 2013) without ΔR correction was used for converting ¹⁴C ages into calendar ages (error is ± 0.2 kyr). Core MD98-2196 is covering over the last

Table 1.Reconstructed pCO2 and SST.

Age	Count	Shell weight (µg)				Shel	Shell diameter (× $10^2 \mu m$)			Size-normalized weight $(\times 10^{-2} \mu\text{g}/\mu\text{m})$			
(куг)	cyr)	mean	SD	max.	min.	mean	SD	max.	min.	mean	SD	max.	min.
3	15	11.7	4.9	23.5	4.6	3.68	0.54	4.75	2.92	3.10	0.90	5.16	1.58
10	23	12.8	3.7	20.7	5.3	4.05	0.59	5.08	2.96	3.13	0.62	4.29	1.58
20	11	14.0	5.4	25.7	8.5	4.01	0.62	4.87	3.28	3.40	0.96	5.38	2.36
30	15	17.9	7.7	29.9	5.8	4.14	0.54	5.41	3.52	4.19	1.44	6.27	1.57
40	17	16.1	5.1	24.9	8.6	4.24	0.53	5.22	3.30	3.76	1.04	6.33	2.56
50	21	15.5	4.5	25.1	6.8	4.27	0.55	5.14	3.12	3.59	0.77	5.13	2.18
60	17	13.8	5.9	27.6	4.3	3.70	0.58	4.69	2.70	3.58	1.10	5.89	1.59
70	18	12.8	3.8	19.9	6.3	3.92	0.58	5.06	3.07	3.23	0.64	4.78	1.97
80	22	12.5	4.8	22.4	6.1	3.95	0.57	5.17	2.91	3.07	0.80	4.94	1.86
90	23	15.7	5.9	26.1	6.6	4.31	0.58	5.37	3.21	3.55	0.94	5.43	2.06
100	23	12.8	4.6	22.6	6.4	4.00	0.50	4.99	3.10	3.12	0.81	5.05	1.96

Table 2. Shell weight, maximum shell diameter and shell size-normalized weight of G. ruber (w). SD, standard deviation.

190 kyr in a time interval from the end of marine isotope stage (MIS) 7 to the Holocene. The average sedimentation rate was 30.4 cm/kyr during glacial periods. On the other hand, during interglacial periods it was 16.9 cm/kyr (Ujiié et al., 2016). For correlation investigation, reported SST (Ujiié et al., 2016) was used (Figure 2A). Concerning past ocean pCO_2 , there is no available reconstructed pH record around the North Pacific including the East China Sea for the last 100 kyr, we utilized a global atmospheric pCO₂ record (Petit et al., 1999) to estimate the variability of the ocean surface pH (Figure 2B, C). Assuming that the total alkalinity was constant during the last 100 kyr, pH was calculated using CO2calc v4. Strictly speaking, the local pH is determined by complicated carbonate chemistry including global and local processes, but we regard this calculated pH as a rough estimation of the past local pH in the East China Sea.

Foraminiferal collection and weight-size measurement

Sediment samples (5–8 cm³) were dried at 40°C for 12 h, and then washed and sieved through a 63 µm mesh. The dried > 63 µm fractions were sieved again through a 250 µm mesh sieve, and all the shells of *G. ruber* (w) were picked from the samples under a stereomicroscope (Olympus SZX12, Olympus Optical Co., Ltd., Tokyo, Japan). The weights of every shell of *G. ruber* (w) were measured individually with a microbalance (Orion Cahn C-35, Thermo Scientific, USA). The analytical precision during the weight measurements was 0.4 µg ($\pm 1\sigma$) (n = 20). Every foraminiferal shell from each sample



Figure 3. Time series of shell parameters (size, weight, volume and density) of *G. ruber* (w) in core MD98-2196. Error bars show standard deviation.

Sample ID	Shell weight (µg)	Shell diameter (×10 ² µm)	Shell volume $(\times 10^{-3} \text{ mm}^3)$	Shell density (mg/mm ³)	Size-normalized weight (× $10^{-2} \mu g/\mu m$)	Size-normalized volume $(\times 10^{-5} \text{ mm}^{3}/\mu\text{m})$
3ka-05	8.2	3.38	4.60	1.78	2.42	1.36
3ka-09	7.8	3.50	4.98	1.56	2.23	1.42
3ka-13	8.0	3.07	4.11	1.94	2.61	1.34
10ka-03	10.0	3.85	5.64	1.77	2.60	1.46
10ka-06	11.4	4.43	7.17	1.59	2.57	1.62
10ka-07	13.1	4.66	7.23	1.81	2.81	1.55
20ka-03	19.9	4.79	10.47	1.90	4.15	2.18
20ka-04	18.6	3.98	9.96	1.87	4.68	2.50
20ka-11	14.4	3.89	7.50	1.92	3.70	1.93
70ka-03	10.5	3.27	5.34	1.97	3.21	1.64
70ka-08	13.4	4.13	7.59	1.76	3.25	1.84
70ka-15	11.2	3.55	5.96	1.88	3.15	1.68
90ka-02	13.5	3.79	7.07	1.91	3.56	1.87
90ka-06	12.8	3.97	6.59	1.94	3.22	1.66
90ka-12	13.2	4.07	6.78	1.95	3.25	1.67
90ka-19	17.1	4.67	8.45	2.02	3.66	1.81
90ka-21	15.0	4.73	7.85	1.91	3.17	1.66
90ka-23	12.2	3.95	7.25	1.68	3.09	1.84
100ka-01	12.9	4.16	7.43	1.74	3.10	1.78
100ka-04	8.7	3.37	4.55	1.91	2.58	1.35
100ka-08	13.3	4.46	7.97	1.67	2.98	1.79

Table 3. Size-normalized weight and MXCT investigations of G. ruber (w).

was photographed with the stereomicroscope for size (i.e., maximum diameter) analysis. Digital images of all the shells were obtained with a digital microscope camera (Olympus E-PL6, Olympus Optical Co., Ltd., Tokyo, Japan), and the maximum diameters of the shells were determined with graphics process software (CorelDRAW X8, Corel Co. Ltd. USA).

For calculating the shell SNW of each specimen, equation 1 was used according to the traditional method (Keul *et al.*, 2013).

w' = w/s....(1)

where w' is the SNW, *s* and *w* are the observed shell size and weight.

Micro-X-ray computed tomography scanning

MXCT scanning was performed at the Center for Advanced Marine Core Research, Kochi University. The high-resolution MXCT ZEISS Xradia 410 Versa (Carl Zeiss X-ray Microscopy, Inc., Germany) was used to observe the internal ultrastructures and density distribution of the shells of *G. ruber* (w) with high-resolution settings (X-ray tube voltage 80 kV, X-ray tube current 125 μ A, resolution 1.0 μ m/pixel, detector array size 1,000 × 1,000 pixels, rotation step 0.18°). MXCT measurements are time-consuming and hence were not performed on all specimens. Thus, from each sample, three to six specimens (in which the SNW value was close to the mean value) were selected for MXCT measurements. To cover the variation range of *p*CO₂, [CO₃^{2–}], and pH over the last 100 kyr, six sample horizons were



Figure 4. Correlations of the size-normalized weight of *G. ruber* (w) in core MD98-2196 with concomitant atmospheric pCO_2 (A) and SST (B). Error bars show standard deviation.

selected from the core MD98-2196 (Table 1). Specimens were glued onto the top of the sample stage, made with a pencil core using water-soluble glue (tragacanth gum) (Appendix 1); therefore, specimens could be recovered afterward. The 3D imaging software MolcerPlus (White Rabbit Corp., Tokyo, Japan) was used for visualizing the 3D CT image.

Measurements of foraminiferal shell volume and density

3D images of each foraminiferal shell were generated based on tiff image stacks made up as pixels. Because the length of one side of the voxel depends on the resolution of MXCT scanning (1.0 μ m in this study), the volume of each voxel can be estimated. The total number of voxels of the whole cell was added up and the total volume was displayed as the MXCT-based shell volume of each foraminiferal shell. Since it is also a size dependent parameter, shell volume was size-normalized similarly to weight. The size-normalized volume (SNV) V' was calculated by the equation 2.



Figure 5. Correlations of the size-normalized shell volume of G. *ruber* (w) with concomitant atmospheric pCO_2 (A) and SST (B).

where V is the shell volume. The shell density ρ was calculated using the equation $\rho = w/V$. Calculations and statistical investigations (Student's t-distribution) were performed in Excel (Microsoft Office 2010).

Results

Shell weight and size measurement

The shell weight and size of 205 *G. ruber* (w) specimens in core MD98-2196 were measured. Table 2 and Figure 3 show the results of the measurements. The shell weight ranged from 4.3–29.9 µg, and the average value was 14.1 µg. The heaviest individual was in a sample at 30 ka, and the maximum average shell weight of each sample was also observed at 30 ka (17.9 µg). The shell size ranged between $2.70 \times 10^2 -5.41 \times 10^2$ µm, the average value was 4.04×10^2 µm, and the largest individual was observed at 30 ka. Conversely, the lightest value was observed at 60 ka (4.3 µg). This sample also included the smallest individual (2.70 × 10² µm). The mean shell size showed more constant values than the mean shell

weight (Figure 3).

The shell weight of *G. ruber* (w) showed higher correlation to each diameter at all samples (Appendix 2; R values were over 0.78 except for the 40 ka sample, which showed *R* (correlation coefficient) = 0.59). The mean value of the SNW (Table 2) peaked at 30 ka with 4.19 × $10^{-2} \mu g/\mu m$. It showed the lowest value at 80 ka (3.07 × $10^{-2} \mu g/\mu m$).

MXCT measurement

In all, 21 specimens were scanned by MXCT, and each of their shell volumes were calculated (Table 3, Figure 3). The smallest shell volume $(4.11 \times 10^{-3} \text{ mm}^3)$ was observed at 3 ka. The other two specimens at 3 ka that were from the same sample also showed relatively smaller shell volume $(4.60 \times 10^{-3} \text{ mm}^3 \text{ and } 4.98 \times 10^{-3} \text{ mm}^3)$. The maximum value of the shell volume $(10.47 \times 10^{-3} \text{ mm}^3)$ was observed at 20 ka, and the other individual from the same sample provided the second largest value (9.96 \times 10^{-3} mm³). Because MXCT can precisely measure each shell volume, it provides precise mean shell densities (Table 3, Figure 3). The shell densities showed a much smaller range (only less than 30% changed) than the other shell parameters: from 1.56-2.02 mg/mm³ (Table 3, Figure 3). This result suggests that the shell density remains relatively constant and is consistent with the higher correlation coefficient between shell volume and weight (R =0.97) throughout the study period.

Statistical results

After these measurements and calculations, correlations between foraminiferal shell parameters (SNW, SNV, and shell density) and environmental parameters (pCO_2 and SST) were tested by using Student's t-distribution. The SNW showed good correlations to such environmental parameters (pCO_2 and SST), the correlation coefficients were R = -0.60 (p = 0.053, n = 11) and R = -0.75(p < 0.01, n = 11), respectively (Figure 4). The p-value of correlation between pCO_2 was not low enough to certify their correlation.

The shell volumes were also used with size-normalized value in a correlation test. It showed higher negative correlation with environmental parameters (Figure 5), R = -0.79 (p < 0.0001, n = 21) with pCO_2 , and R = -0.85 (p < 0.0001, n = 21) with SST. A weak negative correlation of shell density (R = -0.49, p < 0.05, n = 20) was observed with pCO_2 (Figure 6A), this is a significant correlation. However, the slope of the regression line is 0.0022, it showed nearly horizontal line, meaning shell density and SST was insignificant (p = 0.061, n = 20), and it also was constant in figure (Figure 6B). In addition, the higher linear correlation coefficient of shell volume



Figure 6. Correlations of the shell density of *G. ruber* (w) with concomitant atmospheric pCO_2 (A) and SST (B).

and weight (R = 0.97) also suggested that the shell density remains relatively constant through the whole period.

Discussion

Post-depositional alteration

To apply SNW as a proxy for environmental conditions, it is necessary to consider the extent to which foraminiferal shells are preserved in marine sediments (Barker et al., 2004; Gibbs et al., 2010). Above the lysocline, acidification and undersaturated carbonate states of the pore water produced by oxidation of organic material in sediments contribute to carbonate dissolution (Archer and Maier-Reimer, 1994; Martin and Sayles, 1996). Conversely, under high salinity and oversaturated carbonate conditions, the precipitation of secondary calcite on the outer crust of foraminiferal shells may show overestimated SNW with the addition of shell weight (Marshall et al., 2013). Therefore, post-depositional alterations such as partial dissolution or the secondary calcite precipitation of foraminiferal shells should be examined thoroughly before being used to indicate surface seawater carbonate chemistry. In previous studies,



Figure 7. Top views of MXCT sectional models of G. ruber (w) specimens. The shell walls and pores can be clearly observed and some smallest chambers either remained (10, 90 and 100 ka). The color contrast reflects the absorption of X-rays. Scale bar 100 μ m.

several semi-quantitative methods have been developed to evaluate post-depositional dissolution of foraminiferal shells in marine sediments, such as the fragmentation ratio method (Berger, 1970; Metzler et al., 1982; Le and Shackleton, 1992; Mekik and François, 2006). However, post-depositional dissolution of foraminiferal shells often initiates from the inner chambers, it may not be possible to detect the post-depositional alteration by observing only from the outside of the shells (Brown and Elderfield, 1996; Iwasaki et al., 2015). Johnstone et al. (2010) used MXCT to examine the post-depositional dissolution of foraminiferal shells from core sediments. Based on the appearance of MXCT images, the post-depositional dissolution process was divided into five stages. This study also showed that dissolution was initiated from the inner chambers of the shell.

In this study, samples were collected from shallower depth (951 m) than the present depth of the lysocline (~1,600 m; Horikawa *et al.*, 2015). Therefore, expectedly, the post-depositional dissolution on shells of *G. ruber* (w) in the sediments should be very limited. Actually, because these MXCT images (Figure 7) and SEM surface images (Appendix 3) showed that the test wall and the pores appeared distinct and the smallest inner chambers remained, those specimens were classified as a well-preserved sample (Johnstone *et al.*, 2010).

Which shell density or shell wall thickness is reflected in the shell size-normalized weight?

The SNW of planktic foraminiferal shells has been interpreted as a change in either shell density or wall thickness. It is still an unsolved issue as to which parameter the SNW actually represents. Assuming that the foraminiferal shell wall thickness is invariant and independent of growth environmental settings, Broecker and Clark (2002) investigated the SNW of planktic foraminif-



Figure 8. Correlations of the size-normalized weight of *G. ruber* (w) in core MD98-2196 with shell size-normalized volume (A) and shell density (B).

eral shells to estimate the $[CO_3^{2^-}]$ of Atlantic upper deep water. However, Bijma *et al.* (2002) expected that surface seawater $[CO_3^{2^-}]$ would affect shell wall thickness and predicted that SNW is a proxy of this relationship. But they provided no direct evidence.

As a substitute value, we used the MXCT-based shell volume, reflected as the mean shell wall thickness, to examine the relationship between shell wall thickness and SNW. As it is expected from constant density, good correlation between shell SNV and SNW was observed (R = 0.93, Figure 8A). On the other hand, shell density related to SNW, but showed only weak correlation (R =0.48, Figure 8B). However, as there was no clear slope, it suggests constancy of density and that SNW might not track shell density. Therefore, the SNW of G. ruber (w) is mainly controlled by mean shell wall thickness (shell volume) and is less affected by shell density. The effect of size might not have completely disappeared in the validation of the relationship between SNV and SNW. Thus, it is necessary to elucidate a more precise interpretation of SNW and how SNW follows shell volume when shell

Table 4. Correlation between shell parameters (volume, density and size-normalized weight) and environmental parameters (atmospheric pCO_2 and SST). *R* is correlation coefficient and (*) shows non-significance.

	pCO ₂	SST	
Size-normalized volume	$\sqrt{\sqrt{2}}$	$\sqrt{\sqrt{2}}$	
R (p-value)	-0.79 (<0.0001)	- 0.85 (<0.0001)	
Size-normalized weight	$\sqrt{\sqrt{1}}$	$\sqrt{\sqrt{1}}$	
R (p-value)	-0.60 (0.053)*	-0.75 (<0.01)	
Density	\checkmark	\checkmark	
R (p-value)	-0.49 (0.029)	-0.42 (0.061)*	

density changes in the future study such as a laboratory experiment.

Response of planktic foraminiferal shells to changes of pCO_2 and SST

The influence of ocean environmental conditions on planktic foraminiferal calcification rate was investigated by comparing the shell SNW, the shell SNV and the shell density of G. ruber (w) in core MD98-2196 to the variation in atmospheric pCO₂ and SST during the last 100 kyr. The shell SNV of G. ruber (w) was negatively correlated with pCO_2 (R = -0.79, Figure 5A) and SST (R = -0.85, Figure 5B). Seawater temperature has been considered one of the controlling factors on the size variation of foraminiferal shells, with warmer seawaters producing relatively larger shells (Bé et al., 1973; Schmidt et al., 2004). It means that shell weight and volume would get larger with increase in seawater temperature. However, in this study, the SNW and the SNV of G. ruber (w) in core MD98-2196 seemingly showed a negative correlation with SST (Figures 4B, 5B). As SST increased from 20.6 or 20.3 to 26.4°C, despite being within the optimum range of temperature (14-32°C; Bijma et al., 1990), the mean shell SNW and SNV decreased from 4.19 to 3.10 \times $10^{-2} \ \mu g/\mu m$ and from 2.21 to $1.37 \ \times \ 10^{-5} \ mm^{3}/\mu m$, respectively. Such reduction in SNW and SNV may have occurred because the investigated specimens contained the effect of SST with those of many other natural conditions (e.g. carbonate states, food availability, ontogenetic effect, and so on). Particularly on the investigated samples, high temperature environment was a higher pCO₂ environment. Another presented result indicated that higher pCO₂ made their shell SNV lower (Figure 5A), and the SNW also showed negative correlation with pCO₂ although it was not statistically significant (Figure 4A). This result agrees with previous studies of G. ruber (w) (An et al., 2015; Todd et al., 2020) and Globigerina *bulloides* (Iwasaki *et al.*, 2015; Todd *et al.*, 2020) based on laboratory experiments and geological record during the Pliocene, suggesting that ocean acidification would reduce foraminiferal calcification. Therefore, it is suggested that the shell volume (and probably also weight) of *G. ruber* (w) was more influenced by pCO_2 than SST.

Conversely, the foraminiferal shell density showed constant value with each environmental parameter (Figure 6). Concerning SST, constancy of foraminiferal shell density was also reported on larger benthic foraminifer (Kinoshita et al., 2021). These results implied that shell density of G. ruber (w) is less susceptible to the environmental factors, or other environmental factors (e.g. food availability, carbonate chemistry, and life cycles) might affect shell density more than pCO_2 and SST. In any case, foraminiferal shells grown naturally are affected by some growth environmental settings. Owing to its complexity, it is difficult to quantitatively investigate how shells would be influenced by each factor through field research. For this reason, it is imperative to study specimens grown under controlled conditions such as cultured specimens (e.g. Spero et al., 1997).

Comparing the correlations of three shell parameters in our experiment (SNV, density, and SNW) and environmental parameters (pCO_2 and SST), the best parameter tracking pCO_2 and SST was the SNV (Table 4). However, when considering the relationship between SNV and SST, it should be noted that other environmental factors, such as pCO_2 , may have a stronger influence. In all cases, if ongoing global warming and ocean acidification would continue, the carbonate production rate of *G. ruber* (w) will become lower because their shells would become smaller with thinner shell wall.

Conclusions

We measured the maximum diameters and the weight of 205 specimens of G. ruber (w) from core MD98-2196, and investigated 21 specimens by MXCT. The shell volume that was determined using MXCT in addition to traditional size and weight measuring suggested that the environmental changes associated with ocean acidification and global warming will prevent foraminifers from building larger carbonate shells with thick wall. The shell density was not or weakly affected by the variation of SST and atmospheric pCO_2 , it showed constant value for whole investigated samples. Probably it might be affected by another environmental constituent. It is crucial to investigate specimens in which one can compare their growth conditions more quantitatively for demonstrating relationships. Moreover, the SNW of G. ruber (w) shells showed highly significant correlation with shell wall thickness not density. Then, it is interpreted as a tracking parameter of shell wall thickness (mean shell volume). Finally, our results suggest that when using planktic foraminiferal shells as environmental pCO_2 proxies, it is more reasonable to use not only the SNW of the shells but also the SNV. Nevertheless, our results suggested that pCO_2 possibly has more effects on foraminiferal shells, more specific investigation is required of effects of single environmental parameters.

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References

- An, B., Li, T., Sun, H., Xiong, Z. and Chang, F., 2015: Application of Planktonic Foraminifera Size-Normalized Shell Weight in the Western Pacific. *Earth Science*, vol. 24, p. 881–889.
- Archer, D. and Maier-Reimer, E., 1994: Effect of deep-sea sedimentary calcite preservation on atmospheric CO₂ concentration. *Nature*, vol. 367, p. 260–263.
- Barker, S. and Elderfield, H., 2002: Foraminiferal Calcification Response to Glacial-Interglacial Changes in Atmospheric CO₂. *Science*, vol. 297, p. 833–836.
- Barker, S., Kiefer, T. and Elderfield, H., 2004: Temporal changes in North Atlantic circulation constrained by planktonic foraminiferal shell weights. *Paleoceanography*, vol. 19, doi:10.1029/2004pa001004.
- Bé, A. W. H., 1977: An ecological, zoogeographic and taxonomic review of recent planktonic foraminifera. *In*, Ramsay, A. T. S. *ed.*, *Oceanic Micropaleontology, Chapter 1*, vol. 1, P. 1–100. Academic Press, London.
- Bé, A. W. H., Harrison, S. M. and Lott, L., 1973: Orbulina universa d'Orbigny in the Indian Ocean. Micropaleontology, vol. 19, p. 150–192.
- Berger, W. H., 1970: Planktonic foraminifera: selective solution and the lysocline. *Marine Geology*, vol. 8, p. 111–138.

- Bijma, J., Faber, W. W. and Hemleben, C., 1990: Temperature and salinity limits for growth and survival of some planktonic foraminifers in laboratory cultures. *Journal of Foraminiferal Research*, vol. 20, p. 95–116.
- Bijma, J., Hönisch, B. and Zeebe, R., 2002: Impact of the ocean carbonate chemistry on living foraminiferal shell weight: Comment on "Carbonate ion concentration in glacial-age deep waters of the Caribbean Sea" by W. S. Broecker and E. Clark. *Geochemistry*, *Geophysics*, *Geosystems*, vol. 3, doi: 10.1029/2002GC000388.
- Briguglio, A. and Hohenegger, J., 2014: Growth oscillation in larger Foraminifera. *Paleobiology*, vol. 40, p. 494–509.
- Briguglio, A., Kinoshita, S., Wolfgring, E. and Hohenegger, J., 2016: Morphological variations in *Cycloclypeus carpenteri*: Multiple embryos and multiple equatorial layers. *Palaeontologia Electronica*, vol. 19, doi: 10.26879/595.
- Broecker, W. S. and Clark, E., 2001: Glacial-to-Holocene redistribution of carbonate ion in the deep sea. *Science*, vol. 294, p. 2152–2155.
- Broecker, W. S. and Clark, E., 2002: Carbonate ion concentration in glacialage deep waters of the Caribbean Sea. *Geochemistry, Geophysics, Geosystems*, vol. 3, doi: 10.1029/2001GC000231.
- Brown, S. J. and Elderfield, H., 1996: Variations in Mg/Ca and Sr/Ca ratios of planktonic foraminifera caused by postdepositional dissolution: Evidence of shallow Mg-dependent dissolution. *Pale*oceanography and Palaeoclimatology, vol. 11, p. 543–551.
- Caldeira, K. and Wickett, M. E., 2003: Anthropogenic carbon and ocean pH. *Nature*, vol. 425, p. 365–365.
- de Villiers, S., 2004: Optimum growth conditions as opposed to calcite saturation as a control on the calcification rate and shell-weight of marine foraminifera. *Marine Biology*, vol. 144, p. 45–49.
- Eder, W., Wöger, J., Kinoshita, S., Hohenegger, J. and Briguglio, A., 2019: Growth estimation of the larger foraminifer *Heterostegina depressa* by means of population dynamics. *PeerJ*, doi:10.7717/ peerj.6096.
- Gibbs, S. J., Stoll, H. M., Bown, P. R. and Bralower, T. J., 2010: Ocean acidification and surface water carbonate production across the Paleocene–Eocene thermal maximum. *Earth and Planetary Science Letters*, vol. 295, p. 583–592.
- Görög, Á., Szinger, B., Tóth, E. and Viszkok, J., 2012: Methodology of the micro-computer tomography on foraminifera. *Palaeontologia Electronica*, vol. 15, doi:10.26879/261.
- Hemleben, C., Spindler, M. and Anderson, O. R., 1989: Modern planktonic foraminifera, 363 p. Springer-Verlag, New York.
- Hohenegger, J., Kinoshita, S., Briguglio, A., Eder, W. and Wöger, J., 2019: Lunar cycles and rainy seasons drive growth and reproduction in nummulitid foraminifera, important producers of carbonate buildups. *Scientific Reports*, vol. 9: 8286. doi:10.1038/s41598-019-44646-w.
- Horikawa, K., Kodaira, T., Zhang, J. and Murayama, M., 2015: $\delta^{18}O_{sw}$ estimate for *Globigerinoides ruber* from core-top sediments in the East China Sea. *Progress in Earth and Planetary Science*, vol. 2. doi: 10.1186/s40645-015-0048-3.
- Iwasaki, S., Kimoto, K., Sasaki, O., Kano, H., Honda, M. C. and Okazaki, Y., 2015: Observation of the dissolution process of *Globigerina bulloides* tests (planktic foraminifera) by X-ray microcomputed tomography. *Paleoceanography*, vol. 30, p. 317–331.
- Iwasaki, S., Suzuki, A., Iguchi, A., Sasaki, O., Kano, H., Ohno, Y. and Enomoto, K., 2018: Effect of seawater turbulence on formation of coral primary polyp skeletons. *Coral Reefs*, vol. 37, p. 939–944.
- Johnstone, H. J. H., Schulz, M., Barker, S. and Elderfield, H., 2010: Inside story: An X-ray computed tomography method for assessing dissolution in the tests of planktonic foraminifera. *Marine Micropaleontology*, vol. 77, p. 58–70.

Kawahata, H., Fujita, K., Iguchi, A, Inoue, M., Iwasaki, S., Kuroyanagi,

A., Maeda, A., Manaka, T., Moriya, K., Takagi, H., Toyofuku, T., Yoshimura, T. and Suzuki, A., 2019: Perspective on the response of marine calcifiers to global warming and ocean acidification—Behavior of corals and foraminifera in a high CO₂ world "hot house". *Progress in Earth and Planetary Science*, vol. 6, doi:10.1186/s40645-018-0239-9.

- Keul, K., Langer, G., de Nooijer, L. J. and Bijma, J., 2013: Effect of ocean acidification on the benthic foraminifera *Ammonia* sp. is caused by a decrease in carbonate ion concentration. *Biogeosci*ences, vol. 10, p. 6185–6198.
- Key, R. M., Kozyr, A., Sabine, C. L., Lee, K., Wanninkhof, R., Bullister, J. L., Feely, R. A., Millero, F. J., Mordy, C. and Peng, T. H., 2004: A global ocean carbon climatology: Results from Global Data Analysis Project (GLODAP). *Global Biogeochemical Cycles*, vol. 18, doi:10.1029/2004GB002247.
- Kinoshita, S., Eder, W., Wöger, J., Hohenegger, J. and Briguglio, A., 2017: Growth, chamber building rate and reproduction time of *Palaeonummulites venosus* (Foraminifera) under natural conditions. *Coral Reefs*, vol. 36, p. 1097–1109.
- Kinoshita, S., Kuroyanagi, A., Kawahata, H., Fujita, K., Ishimura, T., Suzuki, A., Sasaki, O. and Nishi, H., 2021: Temperature effects on the shell growth of a larger benthic foraminifer (*Sorites orbiculus*): Results from culture experiments and micro X-ray computed tomography. *Marine Micropaleontology*, vol. 163, doi: 10.1016/j. marmicro.2021.101960.
- Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J. P., Langdon, C. and Opdyke, B. N., 1999: Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science*, vol. 284, p. 118–120.
- Kontakiotis, G., Antonarakou, A., Mortyn, P. G., Drinia, H., Anastasakis, G., Zarkogiannis, S. and Möbius, J., 2017: Morphological recognition of *Globigerinoides ruber* morphotypes and their susceptibility to diagenetic alteration in the eastern Mediterranean Sea. *Journal* of *Marine Systems*, vol. 174, p. 12–14.
- Kump, L. R., Bralower, T. J. and Ridgwell, A., 2009: Ocean acidification in deep time. *Oceanography*, vol. 22, p. 94–107.
- Langer, M. R., 2008: Assessing the contribution of foraminiferan protists to global ocean carbonate production. *Journal of Eukaryotic Microbiology*, vol. 55, p. 163–169.
- Le, J. and Shackleton, N. J., 1992: Carbonate dissolution fluctuations in the western equatorial Pacific during the late Quaternary. *Pale*oceanography and Paleoclimatology, vol. 7, p. 21–42.
- Lisiecki, L. E. and Raymo, M. E., 2005: A Pliocene–Pleistocene stack of 57 globally distrib uted benthic δ^{18} O records. *Paleoceanography and Paleoclimatology*, vol. 20, doi:10.1029/2004PA001071.
- Lohmann, G. P., 1995: A model for variation in the chemistry of planktonic foraminifera due to secondary calcification and selective dissolution. *Paleoceanography and Paleoclimatology*, vol. 10, p. 445–447.
- Marshall, B. J., Thunell, R. C., Henehan, M. J., Astor, Y. and Wejnert, K. E., 2013: Planktonic foraminiferal area density as a proxy for carbonate ion concentration: A calibration study using the Cariaco Basin ocean time series. *Paleoceanography*, vol. 28, p. 363–376.
- Marshall, B. J., Thunell, R. C., Spero, H. J., Henehan, M. J., Lorenzoni, L. and Astor, Y., 2015: Morphometric and stable isotopic differentiation of *Orbulina universa* morphotypes from the Cariaco Basin, Venezuela. *Marine Micropaleontology*, vol. 120, p. 46–64.
- Martin, W. R. and Sayles, F. L., 1996: CaCO₃ dissolution in sediments of the Ceara Rise, western equatorial Atlantic. *Geochimica et Cosmochimica Acta*, vol. 60, p. 243–263.
- Mekik, F. and François, R., 2006: Tracing deep-sea calcite dissolution: Agreement between the *Globorotalia menardii* fragmentation index and elemental ratios (Mg/Ca and Mg/Sr) in planktonic fora-

minifers. Paleoceanography, vol. 21, doi:10.1029/2006PA001296.

- Mekik, F. and Raterink, L., 2008: Effects of surface ocean conditions on deep-sea calcite dissolution proxies in the tropical Pacific. *Paleoceanography and Paleoclimatology*, vol. 23, doi:10.1029/2007PA001433.
- Metzler, C. V., Wenkam, C. R. and Berger, W. H., 1982: Dissolution of foraminifera in the eastern equatorial Pacific; an in situ experiment. *Journal of Foraminiferal Research*, vol. 12, p. 362–368.
- Moy, A. D., Howard, W. R., Bray, S. G. and Trull, T. W., 2009: Reduced calcification in modern Southern Ocean planktonic foraminifera. *Nature Geoscience*, vol. 2, p. 276–280.
- Naik, S. S., Naidu, P. D., Govil, P. and Godad, S., 2010: Relationship between weights of planktonic foraminifer shell and surface water CO₃⁼ concentration during the Holocene and Last Glacial Period. *Marine Geology*, vol. 275, p. 278–282.
- Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner, G. K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D., Totterdell, I. J., Weirig, M. F., Yamanaka, Y. and Yool, A., 2005: Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, vol. 437, p. 681–686.
- Osborne, E. B., Thunell, R. C., Marshall, B. J., Holm, J. A., Tappa, E. J., Benitez-Nelson, C., Cai, W. J. and Chen, B., 2016: Calcification of the planktonic foraminifera *Globigerina bulloides* and carbonate ion concentration: Results from the Santa Barbara Basin. *Paleoceanography and Paleoclimatology*, vol. 31, p. 1083–1102.
- Petit, J. R., Jouzel, J., Raynaud, D., Barkov, N. I., Barnola, J. M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotlyakov, V. M., Legrand, M., Lipenkov, V. Y., Lorius, C., Pepin, L., Ritz, C., Saltzman, E. and Stievenard, M., 1999: Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature*, vol. 399, p. 429–436.
- Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P. S., Riebesell, U., Sheperd, J., Turley, C. and Watson, A., 2005: Ocean Acidification due to Increasing Atmospheric Carbon Dioxide, 55 p. The Royal Society, London.
- Reimer, P. J., Bard, E., Bayliss, A., Beck, J. W., Blackwell, P. G., Bronk Ramsey, C., Grootes, P. M., Guilderson, T. P., Haflidason, H., Hajdas, I., HattŽ, C., Heaton, T. J., Hoffmann, D. L., Hogg, A. G., Hughen, K. A., Kaiser, K. F., Kromer, B., Manning, S. W., Niu, M., Reimer, R. W., Richards, D. A., Scott, E. M., Southon, J. R., Staff, R. A., Turney, C. S. M. and van der Plicht, J., 2013: IntCal13 and Marine13 Radiocarbon Age Calibration Curves 0-50,000 Years cal BP. *Radiocarbon*, vol. 55, p. 1869–1887.
- Ridgwell, A. and Zeebe, R. E., 2005: The role of the global carbonate cycle in the regulation and evolution of the Earth system. *Earth and Planetary Science Letters*, vol. 234, p. 299–315.
- Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E. and Morel, F. M. M., 2000: Reduced calcification of marine plankton

in response to increased atmospheric CO₂. *Nature*, vol. 407, p. 364–367.

- Schiebel, R., 2002: Planktic foraminiferal sedimentation and the marine calcite budget. *Global Biogeochemical Cycles*, vol. 16, doi:10.1029/2001GB001459.
- Schiebel, R. and Hemleben, C., 2017: *Planktic Foraminifers in the Modern Ocean*, 358 p. Springer-Verlag, Berlin and Heidelberg.
- Schmidt, D. N., Renaud, S., Bollmann, J., Schiebel, R. and Thierstein, H. R., 2004: Size distribution of Holocene planktic foraminifer assemblages: biogeography, ecology and adaptation. *Marine Micropaleontology*, vol. 50, p. 319–338.
- Speijer, R. P., Van Loo, D., Masschaele, B., Vlassenbroeck, J., Cnudde, V. and Jacobs, P., 2008: Quantifying foraminiferal growth with high-resolution X-ray computed tomography: new opportunities in foraminiferal ontogeny, phylogeny, and paleoceanographic applications. *Geosphere*, vol. 4, p. 760–763.
- Spero, H. J., Bijma, J., Lea, D. W. and Bemis, B. E., 1997: Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes. *Nature*, vol. 390, p. 497–500.
- Todd, C. L., Schmidt, D. N., Robinson, M. M. and De Schepper, S., 2020: Planktic foraminiferal test size and weight response to the Late Pliocene Environment. *Paleoceanography and Paleoclimatology*, vol. 35, e2019PA003738.
- Ujiié, Y., Asahi, H., Sagawa, T. and Bassinot, F., 2016: Evolution of the North Pacific Subtropical Gyre during the past 190 kyr through the interaction of the Kuroshio Current with the surface and intermediate waters. *Paleoceanography and Paleoclimatology*, vol. 31, p. 1498–1513.
- Ujiié, Y., Kimoto, K. and Ishimura, T., 2019: Advanced approach to analyzing calcareous protists for present and past pelagic ecology: Comprehensive analysis of 3D-morphology, stable isotopes, and genes of planktic foraminifers. PLoS ONE, doi:10.1371/journal. pone.0213282.
- Ujiié, Y. and Ujiié, H., 2006: Dynamic changes of the surface and intermediate waters in the Ryukyu Arc region during the past ~250,000 years: based on planktonic and benthic foraminiferal analyses of two IMAGES cores. *Fossils (Palaeontological Society of Japan)*, vol. 79, p. 43–59.

Author contributions

S. K. conducted MXCT data processing and performed the statistical analysis of the data. Q. W. initiated the study and measured morphological datasets. A. K. conducted MXCT data processing. M. M. performed MXCT scanning. Y. U. provided the samples of the core MD98-2196 and the detailed information about these samples. H. K. was supervisor of this study. All authors contributed to the writing of the paper.

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Appendix 1. Experimental settings of MXCT measurements. The sample holder was made of a pencil core, with foraminiferal shell individuals glued onto the top of the sample holder using water-soluble glue (tragacanth gum) so that the shells could be later recovered.



Appendix 2. Correlations of the size and weight of G. ruber (w) in each sample from core MD98-2196.



Appendix 3. SEM images of *G. ruber* (w) specimens in core MD98-2196. In both the full view (A) and the enlarged view (B), the pores are not filled up, and it does not form secondary calcite by deposition.

