1 Differing oxygen isotopic signals of two *Globigerinoides ruber* (white)
2 morphotypes in the East China Sea: Implications for paleoenvironmental
3 reconstructions
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16 ABSTRACT

17 The stable isotope composition of planktonic foraminifera, *Globigerinoides ruber* (*G.*
18 *ruber*), is a commonly used paleoenvironmental proxy. *Globigerinoides ruber* has two primary
19 morphotypes, sensu stricto and sensu lato, which are differentiated by taxonomic criteria and, in
20 some instances, stable isotopic values. Previous studies have reached differing conclusions
21 surrounding the potential bias caused by the use of non-selective mixtures of morphotypes in
22 paleoenvironmental reconstructions involving stable isotopes. Here, *G. ruber* s.s. and *G. ruber*
23 s.l. from five time intervals within the last 85 ka, were analyzed from IODP Site U1429
24 sediments in the East China Sea. The oxygen isotopic compositions of these two morphotypes
25 show statistically significant differences within all five intervals, with smaller s.s. values relative
26 to s.l. values. The largest differences (0.77‰ to 0.82‰) are observed during interglacials
27 (Holocene and Marine Isotopic Stage 5.1) with smaller differences during Marine Isotopic Stage
28 3 (0.45‰ to 0.56‰) and the Last Glacial Maximum (0.21‰). The isotopic offset between s.s.
29 and s.l. is consistent with a deeper (colder) calcification depth of the s.l. morphotypes in the East
30 China Sea. The decreased offset between the two morphotypes with increased glacial conditions
31 may reflect decreased water column stratification during glacial intervals relative to interglacial
32 intervals. According to these results, using a non-selective mixture of *G. ruber* morphotypes in
33 paleoenvironmental reconstructions is inadvisable in this region.
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1. INTRODUCTION

The ubiquitous distribution of planktonic foraminifera in the world's oceans and their geochemistry makes them a powerful tool for paleoenvironmental reconstructions. The stable isotope composition of one species of planktonic foraminifera, *Globigerinoides ruber* (d'Orbigny, 1839), has been used in reconstructing ocean pH (e.g., Hennehan et al. 2013) surface and subsurface seawater temperatures (e.g., Sadekov et al. 2009) as well as changes in the water column structure during the Miocene to Pleistocene (e.g., Wang, 2000).

Globigerinoides ruber (white) has several morphotypes that differ from the original definition of the species *G. ruber* sensu stricto (s.s.) (e.g., Srinivasan et al. 1983). These morphotypes are generally referred to as *G. ruber* sensu lato (s.l.). A number of studies have investigated the potential for bias caused by the use of non-selective mixtures of these morphotypes in the geochemical analyses commonly used for paleoenvironmental reconstructions.

Studies in the Atlantic Ocean (Gulf of Mexico), eastern Indian Ocean, and the central tropical Pacific Ocean found that morphotypical variability has no statistically significant impact on the stable oxygen isotopic composition of *G. ruber* (Thirumalai et al. 2014, Mohtadi et al. 2009, and Lynch-Stieglitz et al. 2015) In contrast, other studies in the Atlantic Ocean (Gulf of Mexico), South China Sea and North Pacific found statistically significant differences between the stable isotopic compositions of the s.s. and s.l. morphotypes (Antonarakou et al. 2015, Kawahata, 2005; Löwemark et al., 2005; Steinke et al., 2005; Wang, 2000).

These differing conclusions imply regional/ocean basin variations or temporal variations in the geochemistry of *G. ruber*. The purpose of this study is to assess the morphological variations of *G. ruber* in the East China Sea, a marginal basin in the western Pacific. This site is currently being used to reconstruct changes in Yangtze River runoff using the isotopic composition of *G. ruber*. Understanding the extent to which s.s. and s.l. must be differentiated is crucial to accurately reconstruct the $\delta^{18}\text{O}$ of sea water. Here, we assess the isotopic compositions of *G. ruber* at five distinct time intervals; Marine Isotope Stage (MIS) 1 (Holocene), MIS 2 (full glacial), MIS 3.1 (stadial and interstadial), MIS 3.3 (stadial and interstadial), and MIS 5.1 (full interglacial). Each marine isotope stage represents distinct regional (and global) environmental conditions. Understanding whether the isotopic compositions vary between morphotypes at this

location and across these climatically distinct time intervals will help to further delineate the geographic nature and potential causes of these differences.

2. MATERIALS AND METHODS

2.1 Location

The samples used for this study were obtained during Integrated Ocean Drilling Program (IODP) Expedition 346 to the East China Sea (Figure 1). The three holes drilled at Site U1429 (31°37.04'N, 128°59.85'E, 732 meters below sea level) were used to produce a composite record of 181.4 meters in length. Our samples are all from within the upper 50 meters.

2.2 Sample Selection

The *Globigerinoides ruber* variants are defined using the taxonomic criteria described by Wang (2000). The s.s. morphotype has symmetrical, spherical chambers with a wide, high-arched aperture (Figure 2A). The s.l. morphotype analyzed in this study has a more compact test with a compressed and flattened final chamber, and a relatively small aperture (Figure 2B). A third morphotype, informally designated as sensu lato extreme (s.l.e.) was differentiated based on its extremely flattened and asymmetrical final chamber and very small aperture (Figure 2C).

Structure in the full (180 meter) planktonic and benthic $\delta^{18}\text{O}$ record (not shown in Figure 3) allowed us to confidently identify all marine isotopic stages (MIS; Prell et al., 1986) spanning the past 380 kybp. Figure 3 shows our sample locations in this context; samples taken from 0.78 – 1.98 meters are from MIS 1 (Holocene), 12.14-14.54 meters from MIS 2 (glacial), 17.90 – 22.10 meters from MIS 3.1 (stadial and interstadial), 26.91 – 29.01 meters from MIS 3.3 (stadial and interstadial), and lastly, samples from 45.10 – 45.55 meters from MIS 5.1 (interglacial).

2.3 Sample Preparation and Stable Isotope Analyses

Sediment samples were wet sieved to remove the fine fraction (<63 μm), dried overnight in a 40°C oven, then dry sieved before isolating the s.s., s.l., and s.l.e. specimens (250-355 μm size fraction) using a binocular microscope. The number of specimens in each sample varied from 3 to 52 with an average of 20 specimens in each sample. Samples with 15 or fewer specimens were cleaned in ethanol and run whole through the mass spectrometer. Samples with

greater than 15 specimens were cleaned in ethanol, crushed, homogenized, and subsampled (~80 micrograms of CaCO_3) prior to running on the mass spectrometer. A total of 96 measurements (s.s., s.l., and s.l.e.) were performed at Brown University using a Finnigan MAT 252 coupled to Kiel III Carbonate Device. The machine precision is $\pm 0.06\text{‰}$ (1σ) for $\delta^{18}\text{O}$ measured on the in-house standards (Carrara Marble and Brown Yule Marble). The stable oxygen isotope results (Table 1) are reported relative to the Vienna Pee Dee Belemnite (VPDB) standard. One s.s. outlier ($\delta^{18}\text{O}$ at 14.24 m) was rejected from the statistical analysis due to insufficient voltage (not enough gas generated to achieve reliable counting statistics).

2.4 Statistical Evaluation

A null hypothesis test was employed to test if the differences between the isotopic compositions of the three morphotypes are statistically significant. The null hypothesis, morphological variability has no effect on *G. ruber* geochemistry, was tested for samples from each time interval using Welch's unpaired T Test (for normally distributed data) and the unpaired Mann Whitney Wilcoxon Ranksum Test (for nonparametric data) following the statistical methodology of Thirumalai et al. (2014). Neither of these tests assume equal sample size or variance. All populations were found to be normally distributed based on the Shapiro-Wilk test, except for the $\delta^{18}\text{O}$ MIS 2 (glacial) samples. All measurements were assessed at the 95% confidence interval ($p < 0.05$). Hence, if the reported p value is greater than this threshold, the null hypothesis cannot be rejected. If the reported p value is less than this threshold, the null hypothesis must be rejected. These analyses were done twice, first comparing the s.l. and s.l.e. variants, then comparing s.s. to the combined s.l. and s.l.e. variants. The results of these statistical analyses are reported in Table 2.

2.5 Predicted $\delta^{18}\text{O}$

We gathered temperature and salinity data from the Japan Oceanographic Data Center for the location of Site U1429. Using the Horikawa et al. (2015) equation, we converted salinity to the oxygen isotopic composition of seawater (Eq.1). This equation was derived from data collected from the Yellow Sea- East China Sea and Kuroshio sites which are in close proximity to Site U1429. The Bemis et al. (1998) highlight equation was used to convert the temperature and $\delta^{18}\text{O}$ of seawater to predicted $\delta^{18}\text{O}$ of calcite (Eq. 2). This $\delta^{18}\text{O}_\text{C}$ – temperature equation was

determined from culturing experiments of planktonic foraminifera species grown under high-light conditions. These equations were employed to replicate the methodology used by Kubota et al. (2010) in the northern East China Sea. Similar methodologies were also used by Khider et al. (2011) and Schmidt (1999).

$$\delta^{18}\text{O}_{\text{sw}} = -7.74(\pm 0.4) + 0.23(\pm 0.1) \times \text{salinity} \quad (\text{Equation 1})$$

$$T(^{\circ}\text{C}) = 14.9 - 4.8(\delta^{18}\text{O}_{\text{C}} - (\delta^{18}\text{O}_{\text{sw}} - 0.27\text{‰})) \quad (\text{Equation 2})$$

3. RESULTS

When analyzing the stable oxygen isotopic oxygen results we found that, across all time intervals, the null hypothesis could not be rejected when comparing the s.l. and s.l.e. morphotypes. In other words, there is no statistically significant difference between the oxygen isotope values of the s.l. and s.l.e. morphotypes. On this basis, the s.l. and s.l.e. morphotypes were combined and compared to s.s., finding that the null hypothesis must be rejected, i.e. there is a statistically significant difference, for all time intervals (Table 2). Wang (2000) also combined the s.l. and s.l.e. sub-groups (referred to as “low and high trochospires”) after finding they did not show significant differences in their isotopic signals. Generally, *G. ruber* s.s. shows lighter $\delta^{18}\text{O}$ values compared to s.l. (Figure 4).

4. DISCUSSION

Three possible mechanisms could explain why the s.s. and s.l. morphotypes show consistently different oxygen isotopic compositions: different seasonal production rates, different calcifying depths, or vital effects.

4.1 Seasonal Production Rate

If seasonal preference is the underlying factor behind this difference, we would expect one morphotype to be more abundant than the other in a particular season, since the heavier $\delta^{18}\text{O}$ values would indicate that s.l. calcifies in colder water while s.s. calcifies in warmer waters (Berger, 1970; Hecht, 1972, Kuroyanagi and Kawahata, 2004). Sediment trap data can be used to

quantify the monthly production of each morphotype. In the previous studies of *G. ruber* morphotypes done by Thirumalai et al. (2014) and Wang (2000) in the Gulf of Mexico and the Pacific Ocean, sediment trap data was used to rule out a seasonal preference. Other studies of these morphotypes either did not have sediment trap data or did not report the flux of the s.s. and s.l. morphotypes separately (Kawahata, 2005; Mohtadi et al., 2009; Steinke et al., 2005). No sediment trap data comparing s.s. to s.l. are available for the East China Sea proper.

Wang et al. (1999) used ten well-dated sediment records with millennial to bidecadal time resolution over the last 220,000 years to conclude that the Last Glacial Maximum had greater seasonality. If these morphotypes have different seasonal production rates, we would expect the offset between their isotopic compositions to increase during times of increased seasonality. The isotopic difference between s.s. and s.l. is smallest during glacial periods and largest during interglacial periods (Table 3). Hence, it is unlikely that a seasonal preference is the mechanism controlling the isotopic composition of these morphotypes. However, these findings by Wang et al. (1999) may be location specific and may not necessarily be applicable to the East China Sea.

4.2 Calcification Depth and the Depth of the Mixed Layer

If these morphotypes have different calcifying depths, we would expect the water column profiles of temperature and salinity to corroborate the measured $\delta^{18}\text{O}$ values. In the western Gulf of Mexico, Thirumalai et al. (2014) use a model to predict the isotopic composition of *G. ruber* shells as a function of season and calcification depth. They found that s.s. and s.l. have similar calcifying depths corroborating their conclusion that s.s. and s.l. are statistically indistinguishable. Wang (2000) and Löwemark et al. (2005) came to the opposite conclusion in the South China Sea. Using in situ water column profiles, they found a 2-3°C temperature decrease and a 0.20-0.25 salinity increase between the surface (0-30 meters) and the subsurface waters (30-50 meters). This is consistent with their observed 0.4‰ average offset between s.s. and s.l. Wang (2000) and Löwemark et al. (2005) inferred that in the South China Sea s.s. inhabits the upper 30 meters of the water column while s.l. lives between 30 and 50 meters. In the eastern Gulf of Mexico, Antonarakou et al. (2015) also found that s.s. inhabits the upper ~30m of the water column while s.l. inhabits depths below 30m.

In the East China Sea, very close to Site U1429, Horikawa et al. (2015) used measured temperature and salinity data to simulate the oxygen isotopic composition of planktonic foraminifera as a function of depth (Eq.1) using the Bemis et al. (1998) calcite equilibrium equation (Eq.2). Applying the same approach to compare the predicted isotopic values with our observed isotopic data, we found that the average Holocene isotopic compositions for s.s. and s.l. (-2.39‰ and -1.62‰ respectively, Table 3) occur at depths of about 10 meters and 60 meters (Figure 5). These results are consistent with the hypothesis that calcifying depth may account for the isotopic offset between the s.s. and s.l. morphotypes.

The East China Sea is a very dynamic region of the ocean. Water column dynamics are impacted by seasonally varying atmospheric conditions such as heating, cooling, and wind stress (Chu et al. 2005) as well as changes in the Kuroshio Current (Andres et al. 2015). Strong regional variation in atmospheric and oceanic conditions may account for differences in depth habitat inferred for our East China Sea data relative to that inferred for the South China Sea (Wang 2000; Löwemark et al., 2005). Regional differences are evident in the extent of isotopic offset observed among s.s. and s.l. morphotypes (Table 4). Previous studies in the South China Sea found isotopic offsets between 0.27‰ and 0.5‰, while in the East China Sea we found offsets are large as 0.77‰ (Table 3). The general trend in the North Pacific marginal basins evaluated to date, appears to be a north to south decrease in the isotopic offset between morphotypes (Figure 1, Table 4). This north to south gradient parallels the north to south deepening of the mixed layer in the summer months when the flux of *G. ruber* is the greatest (Figure 6) (Xu et al. 2005). In the summer months, runoff from the Yangtze River causes increased water column stratification at Site U1429 (Site 1 on Figure 6), while the sites further south are less affected (Hao et al. 2012). We hypothesize that the s.s. depth of calcification is controlled by the depth of the seasonal thermocline while the s.l. depth of calcification is controlled by the permanent thermocline. Regions with increased stratification have greater differences between their seasonal and permanent thermocline depths resulting in a greater $\delta^{18}\text{O}$ offset between s.s. and s.l. Sites further south are less affected by the summer monsoon runoff, resulting in diminishing offsets between their s.s. and s.l. isotopic values.

4.3 Vital Effects

The s.s and s.l. morphotypes have been identified as distinct genotypes of *G. ruber* (Aurahs et al. 2011, Kuroyanagi et al. 2008), consistent with differing isotopic signals. Planktonic foraminifera may not calcify in oxygen isotopic equilibrium with the surrounding seawater, a phenomenon known as ‘vital effects’. The photosynthetic activity of algal symbionts living in the shells of foraminifera alters their calcification rate (Lee et al. 1987, Nooijer et al. 2009), causing the *G. ruber* $\delta^{18}\text{O}$ of calcite and seawater to differ by about ± 0.0 to -1.0% (Niebler et al. 1999). We cannot assess if or how these algal symbionts affect the morphotypes of *G. ruber* or if that difference is a reflection of their differing biology, so we cannot exclude the possibility that vital effects differ among these morphotypes, contributing to their isotopic offset.

4.4 Extent of Glaciation and Changes in the Thermocline

Our results indicate that the extent of glaciation is strongly coupled to the oxygen isotopic offset of the s.s. and s.l. morphotypes (Figure 7). Wang (2000), Kawahata (2005), and Antonarakou (2015) also observed this coupling in their studies of the South China Sea, Northern Pacific, and eastern Gulf of Mexico respectively. In glacial periods, the water column is likely to be less stratified due to colder air temperatures and increased monsoonal wind mixing, driving the seasonal thermocline deeper (Wang, 2000). This results in the permanent and seasonal thermoclines being closer together and the s.s. and s.l. morphotypes having closer $\delta^{18}\text{O}$ values. In interglacial periods, increased runoff drives the seasonal thermocline to shallower depths while the permanent thermocline remains unchanged, resulting in a larger isotopic offset between s.s. and s.l.

Antonarakou (2015) in the eastern Gulf of Mexico also attributes the fluctuations in isotopic offsets to climatic changes, yet found the opposite correlation. Their maximum oxygen isotopic offset corresponds to cold events when the mixed layer is shallowest while the minimum offset corresponds to warm events. Clearly, how water column dynamics respond to climatic events is location specific, but it is evident that the depth of the thermocline is coupled to the extent of glaciation.

4.5 Isotopic Offset and Changing Climatic Conditions

The extent of changing climatic conditions appears to influence the detection of an isotopic offset. When analyzing data across glacial and interglacial boundary conditions the

oxygen isotopic offset between the s.s. and s.l. morphotypes becomes evident. This study, along with Antonarakou et al. 2015, analyzed data from the last glacial event through the Holocene and found a statistically significant difference between the s.s. and s.l. morphotypes. While studies that focused on modern data found differing degrees of isotopic offsets that vary regionally (Table 4).

This may explain why two studies in the Gulf of Mexico found differing results. When looking at modern data, Thirumalai et al. (2014) in the western Gulf of Mexico found no isotopic difference between these morphotypes, while Antonarakou et al. (2015) in the eastern GOM found minor isotopic differences when analyzing the same time frame. When Antonarakou et al. (2015) looked through the late glacial, the isotopic differences reached their maximum values.

We hypothesize that the depth habitat of *G. ruber* morphotypes is influenced by the depth of the mixed layer. Changes in the mixed layer depth are better expressed across glacial and interglacial boundary conditions, and thus more easily detectable.

5. CONCLUSION

This study of the East China Sea as well as five others throughout the Atlantic and Pacific Oceans (Antonarakou 2015, Kawahata, 2005; Löwemark et al., 2005; Steinke et al., 2005; Wang 2000) conclude that morphological variations of the *G. ruber* species result in different isotope compositions. These isotopic differences are most evident when analyzing data over glacial/interglacial conditions. The correlation between our measured Holocene oxygen isotopic compositions and the predicted composition within the water column indicates that the observed isotopic offset is consistent with the morphotypes occupying different calcification depths. This hypothesis is supported by the downcore oxygen isotopic compositions having a smaller offset in the glacial period, when the water column is less stratified, and a larger offset in the interglacial periods, reflecting a more stratified water column. Regionally varying water column stratification may also explain the difference in regional calcification depths in the Holocene, as well. Using a non-selective mixture of morphotypes invites error and possible misinterpretations in paleoenvironmental reconstructions if the region has not been previously assessed for potential differences in morphotype calcification. On the other hand, understanding how the oxygen isotopic composition of s.s. and s.l. relate to the spatial and temporal stratification of the modern water column could lead to a useful means to reconstruct past changes in ocean stratification.

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 396

500 μm

A

500 μm

B

500 μm

C