

RESEARCH ARTICLE

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Key Points:

- Two coexisting genotypes of *Globigerina bulloides* in the Arabian Sea
- Both stable oxygen isotope and Mg/Ca compositions of individual *G. bulloides* shells have bimodal distributions
- Genetic variability affects foraminiferal geochemistry

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Geochemical imprints of genotypic variants of *Globigerina bulloides* in the Arabian Sea

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Abstract Planktonic foraminifera record oceanic conditions in their shell geochemistry. Many palaeoenvironmental studies have used fossil planktonic foraminifera to constrain past seawater properties by defining species based on their shell morphology. Recent genetic studies, however, have identified ecologically distinct genotypes within traditionally recognized morphospecies, signaling potential repercussions for palaeoclimate reconstructions. Here we demonstrate how the presence of *Globigerina bulloides* cryptic genotypes in the Arabian Sea may influence geochemical signals of living and fossil assemblages of these morphospecies. We have identified two distinct genotypes of *G. bulloides* with either cool water (type-II) or warm water (type-I) temperature preferences in the Western Arabian Sea. We accompany these genetic studies with analyses of Mg/Ca and stable oxygen isotope ($\delta^{18}\text{O}$) compositions of individual *G. bulloides* shells. Both Mg/Ca and $\delta^{18}\text{O}$ values display bimodal distribution patterns. The distribution of Mg/Ca values cannot be simply explained by seawater parameters, and we attribute it to genotype-specific biological controls on the shell geochemistry. The wide range of $\delta^{18}\text{O}$ values in the fossil assemblage also suggests that similar controls likely influence this proxy in addition to environmental parameters. However, the magnitude of this effect on the $\delta^{18}\text{O}$ signals is not clear from our data set, and further work is needed to clarify this. We also discuss current evidence of potential genotype-specific geochemical signals in published data on *G. bulloides* geochemistry and other planktonic foraminiferal species. We conclude that significant caution should be taken when utilizing *G. bulloides* geochemistry for palaeoclimate reconstruction in the regions with upwelling activity or oceanographic fronts.

1. Introduction

Reconstruction of past oceanic and climatic conditions relies heavily upon the geochemical signatures found within calcitic shells of planktonic foraminifera [Elderfield and Ganssen, 2000]. Stable isotopic and elemental composition of foraminiferal shells reflect the physio-chemical environment in which they are formed. In order to reconstruct past changes in oceanic conditions using geochemical data from foraminiferal calcite, it is important to obtain a thorough understanding of the relationship between foraminiferal ecology and shell geochemistry. This relationship is based on the assumption that each foraminiferal morphospecies represents a genetically continuous species with a unique habitat preference. As molecular genetic characterization of planktonic foraminifera has progressed, it has become clear that the use of the morphospecies concept may be unsafe. Many cryptic genetic types (or genotypes) have been recognized within modern planktonic foraminiferal morphospecies which have distinct biogeographic distributions related to trophic regimes [de Vargas et al., 1999; Sears et al., 2012] or surface water properties [de Vargas et al., 2001; de Vargas et al., 2002; Darling et al., 2004; Darling et al., 2007; Darling and Wade, 2008; Sears et al., 2012]. Many morphologically defined species therefore represent a mixed assemblage of genotypes [Kucera and Darling, 2002]. Some of these genotypes can be distinguished in fossil assemblages by using subtle differences in their shell morphology. For example, it has been shown that *Orbulina universa* genotypes have

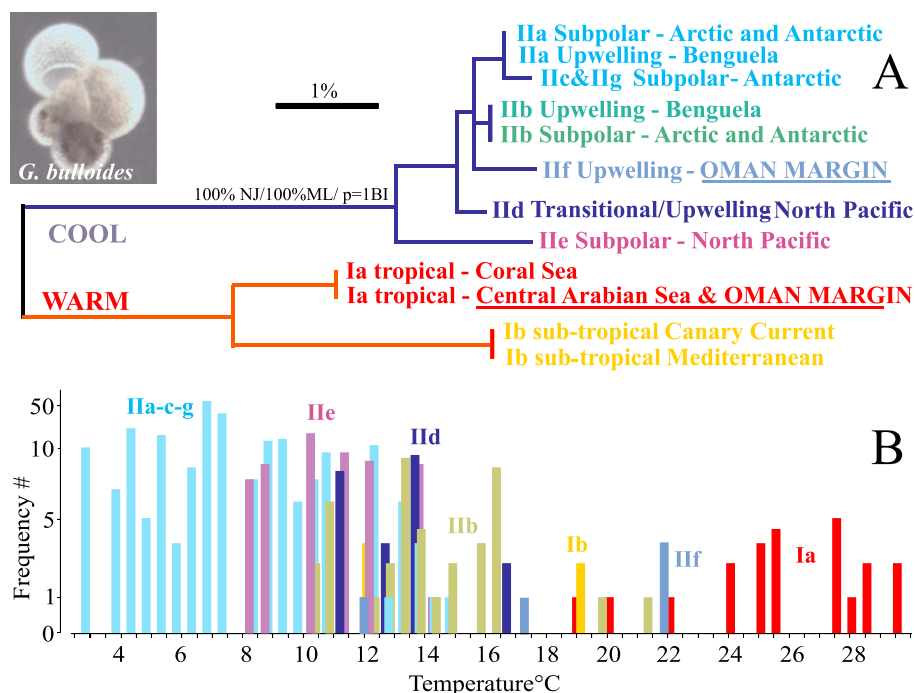


Figure 1. Genetic variability and ecological preferences of *Globigerina bulloides*. (a) Maximum likelihood phylogenetic tree showing the evolutionary relationships among the *G. bulloides* genotypes. The tree is based on 669 nucleotides of the SSU rRNA gene and is rooted on the subtropical *G. bulloides* Type I genotypes. The boot strap value (expressed as a percentage) indicates the high level of support for the separation of the warm and cool water genotypes. The scale bar corresponds to a genetic distance of 1%. (b) Temperature adaptations of *G. bulloides* genotypes based on global databases of ~750 genetic sequences from Darling and Wade [2008] and Morard et al. [2013]. Labeling indicates the different *G. bulloides* genotypes (see Figure 1a). Warm water lineage genotypes are coloured toward the red spectrum and the cool water lineage genotypes toward the blue. Note the temperature-specific range of genotypes and the wide-ranging difference between the ecological adaptations of the coolest IIa and the warmest Ia.

different shell thickness and porosities [Morard et al., 2009] and some members of the *Globigerinoides ruber* genotype cluster can be morphologically split by using trochospiral coiling height and aperture shape [Aurahs et al., 2011; Spezzaferri et al., 2015]. In contrast, other genetically diverse morphospecies such as *Globigerina bulloides* appear to have genotypes with morphologically similar shells [Darling et al., 2007].

How the presence of cryptic genotypes within planktonic foraminifera affects our ability to utilize their shell geochemistry for past climate reconstructions still remains to be answered. Specific geochemical signatures have been reported for some morphotypes of *Globigerinoides ruber* [Steinke et al., 2005; Sadekov et al., 2008; Antonarakou et al., 2015], which also have been identified as different genotypes [Aurahs et al., 2011]. Generally, it has been assumed that such geochemical differences are related to their divergent ecological preferences rather than to variation in the way they incorporate stable isotopes and trace metals into their shell calcite (i.e., genotype specific vital effect) [Numberger et al., 2009; Morard et al., 2016]. If this assumption is correct, then an ecological understanding of the divergent genotypes alone within a planktonic foraminiferal assemblage could prove particularly informative for paleoceanographic studies. However, the possibility still remains that genotypes may also express genotype specific geochemical vital effects. If this turns out to be the case, genotype-specific geochemical calibrations would be required to avoid potential artefacts in reconstructions of past climate.

Globigerina bulloides is one of the most commonly used planktonic foraminifera species in palaeoclimate reconstructions. It occurs in great abundance in the moderately cool temperate to high latitudes; yet it also dominates foraminiferal assemblages within tropical upwelling systems, such as the Western Arabian Sea [Kleijne et al., 1989; Naidu and Malmgren, 1996]. Since *G. bulloides* inhabits such a wide range of different ecosystems, it is not surprising that several ecologically distinct small-subunit (SSU) rRNA genotypes have been recognized within this morphospecies (Figure 1a) [Darling and Wade, 2008; Sears et al., 2012; Morard et al., 2013]. Phylogenetic analysis indicates that the *G. bulloides* genotype complex splits principally into two major groups (Type I and Type II) with significantly divergent ecologies [Darling and Wade, 2008]. To date, the Type I

genotypes (Ia and Ib) have been found in association with warm tropical/subtropical regions and the Type II genotypes (Types IIa–IIe and IIg) with the cooler transitional and high-latitude regions (Figures 1a and 1b). There is a considerable degree of genetic distinction between the two major *G. bulloides* ecological complexes (Type I and Type II). Such deep division suggests that their divergence must have occurred a substantial period of time ago. Recent species delineation studies indicate that the division is well above species level, since a species status is supported for several of the *G. bulloides* genotypes in both complexes [Andre *et al.*, 2014].

In this work, we investigate the relationship between the shell geochemistry of two ecologically distinct (Figure 1b) genetic variants of *Globigerina bulloides*, the most challenging morphospecies among the planktonic foraminifera. We use sediment samples and plankton net samples collected in the Arabian Sea to better understand the effect of *G. bulloides* cryptic genotypes in interpreting the Mg/Ca and $\delta^{18}\text{O}$ signatures of their shells.

2. Material and Methods

2.1. Genetic Study of *G. bulloides* in the Arabian Sea

To investigate the genetic variability of *G. bulloides* in the Arabian Sea, we collected samples during period of upwelling in the western Arabian Sea and also nonupwelling intervals during expeditions on FS Meteor (September 2007; M74/1b) and RRV Charles Darwin (July 2003, CD 148) in the region (Figure 2a). Specimens taken during cruise M74/1b were collected at 15 stations along the cruise track (stations 943–951). Both shallow and deep plankton hauls were carried out at each station accompanied by conductivity, temperature and depth (CTD) measurements, using a multiplankton sampler (Hydro Bios, Kiel, 50 × 50 cm opening, 100 μm mesh size). Two sets of five depth intervals were used for sampling (0–100 m and 0–700 m). Specimens of *G. bulloides* collected during cruise CD 148 are based on filtering the ships' nontoxic water supply through a plankton screen (83 μm mesh; pump inlet at 5 m depth). Sea surface temperature and salinity were logged for each station (S2–S5; Figure 2a). Data and material from RRV Charles Darwin (CD 148) have been previously published in Darling and Wade [2008] and Sears *et al.* [2012].

The *G. bulloides* specimens used for genetic analysis were individually picked, digitally imaged, and transferred into 1.5 mL microtubes with 25 μL of deoxycholate buffer [Holzmann and Pawlowski, 1996] and incubated for 1 h at 60°C. Amplification by polymerase chain reaction and automated sequencing of an ~1000 base pair region of the terminal 3' end of the SSU rRNA gene were as described previously [Darling *et al.*, 2000].

Partial SSU rDNA sequences were aligned manually within version 2.2 of the Genetic Data Environment package [Smith, 1994]. A maximum likelihood phylogenetic tree (employing a general time reversible model and incorporating gamma-distributed rate-heterogeneity between sites) was constructed within Paup* version 4.0d64 [Swofford, 2003]. Six hundred sixty-nine unambiguously aligned nucleotide sites were utilized in the phylogenetic analysis, and the phylogeny was rooted on the subtropical *G. bulloides* Type I genotypes. Bootstrap resampling (1000 replicates) was employed to assign support to branches in the trees [Felsenstein, 1985]. The *G. bulloides* IIc sequence shown in this study (Figures 1a and 1b) is deposited in GenBank under the accession number KX576651.

2.2. Geochemical Studies of *G. Bulloides* in the Arabian Sea

Two microanalytical approaches were used to characterize Mg/Ca and $\delta^{18}\text{O}$ compositions of individual shells of *G. bulloides* from the Arabian Sea.

The laser ablation inductively coupled plasma–mass spectrometry (LA-ICP-MS) at the Department of Earth Science at University of Cambridge was used to measure Mg/Ca ratios of foraminiferal shells from plankton samples collected during FS Meteor cruise M74/1b. Seventy-five shells of *G. bulloides* were measured for Mg/Ca composition. All shells were collected from the top 20 m of the water column using the single multinet (sampling station –949: 16°26'N, 61°15'E; Figure 1) equipped with a net with a mesh width of 150 μm . The LA-ICP-MS system employs an Analyte G2 excimer laser (Teledyne Photon Machines Inc) coupled with a Thermo i-CapQ ICPMS to measure trace metal profiles through foraminiferal shell walls. Sample preparation and analyses were carried out following protocols discussed in Eggins *et al.* [2003] and Sadekov *et al.* [2008]. The horizontal and vertical resolution of the technique was optimized by ablating small-diameter spots (30 × 30 μm) at four laser pulses per second and 1.6 J/cm² laser fluence. Between three and four profiles were generated for

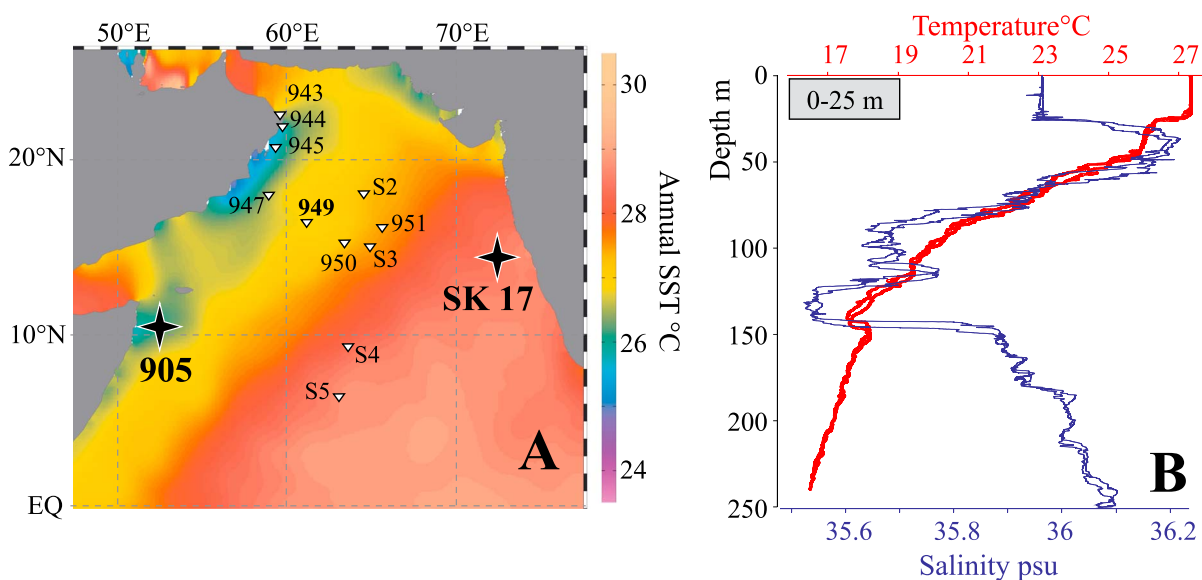


Figure 2. (a) Location map of sampling stations used for geochemical and genetic studies of the *G. bulloides* populations in the Arabian Sea. Stars are core-top samples from sediment cores in the Western and Eastern Arabian Sea; triangles are plankton net samples from FS Meteor (943–951) and RRV Charles Darwin (S2–S5) cruises. Background colours are mean sea surface temperatures from the WOCE data set [Boyer *et al.*, 2013]. (b) Temperature and salinity CTD profiles through the top 250 m of the water column during collection of *G. bulloides* shells at station 949, which were used for Mg/Ca measurements. Note narrow ranges of both temperature and salinity signals for the top 20 m of the water column.

each foraminiferal shell (i.e., one profile for each chamber). The ICP-MS is optimized by using NIST610 reference glass material for maximum sensitivity across Li-Sr mass range and maintaining ThO/Th <0.5% and Th/U ratio ~1. The isotopes ^{11}B , ^{24}Mg , ^{32}S , ^{43}Ca , ^{44}Ca , ^{55}Mn , ^{63}Cu , ^{66}Zn , ^{88}Sr , ^{138}Ba , and ^{27}Al were measured during each depth profile analysis, which required only 20–50 s to acquire. Data reduction involved initial screening of spectra for outliers, subtraction of the mean background intensities (measured with the laser turned off) from the analyzed isotope intensities, internal standardization to ^{43}Ca , and external standardization using the NIST-SRM610 glass reference material. The signals of Al, Mn, Zn, Cu, and Ba were used to distinguish surface contamination from foraminiferal calcite following Sadekov *et al.* [2008]. In-house calcitic standards eBlue was used to monitor long-term Mg/Ca reproducibility, which was 3.63 ± 0.17 mmol/mol (2 SD).

The $\delta^{18}\text{O}$ of *G. bulloides* shells was measured by using the continuous-flow isotope ratio mass spectrometry system at Hokkaido University, which enabled us to determine the $\delta^{18}\text{O}$ for microvolume carbonate samples (as low as 0.2 μg , which is equal to 0.5 of a *G. bulloides* chamber) with high precision and accuracy [Ishimura *et al.*, 2004; Ishimura *et al.*, 2008]. The standard deviations from the analysis of NBS 19 (international standard reference calcite) analyzed on the same day as the samples was $\pm 0.06\text{‰}$ for $\delta^{18}\text{O}$ ($n=6$). All data are reported in standard δ notations ($\delta^{18}\text{O}$; ‰) relative to the Vienna Peedee belemnite standard scale. The weight of the analyzed shells was calculated by using the volume of CO_2 gas evolved during the reaction between calcite and phosphoric acid [Ishimura *et al.*, 2004]. Although the continuous-flow isotope ratio mass spectrometry system at Hokkaido University is designed for very low volume samples, in reality combining Mg/Ca and $\delta^{18}\text{O}$ measurements on samples from plankton nets without compromising quality of both analyses remains analytically challenging. The weight of *G. bulloides* shells used for Mg/Ca analyses range from 0.1 to 2 μg , which is further reduced to 0.05–0.5 μg after sample preparation and laser ablation analyses. Therefore, we used core-top samples for studying variability of $\delta^{18}\text{O}$ in the *G. bulloides* population in the Arabian Sea. Fifty-five shells from the core-top of sediment core SK17 were used for measuring oxygen isotope compositions of the *G. bulloides* assemblage. Core SK17 (15°15'N, 72°58'E, water depth 840 m) was collected in the Eastern Arabian Sea during research cruise ORV Sagar Kanya in 1999 (Figure 2a). *G. bulloides* shells were picked from the 300–350 μm size fraction. Each shell was individually cleaned by ultrasonication in methanol for 1–2 min and then rinsed in MilliQ water.

Using sediment sample archives also allows us to combine our $\delta^{18}\text{O}$ results with data from Ganssen *et al.* [2011], in which a similar analytical approach was performed in the Western Arabian Sea. Ganssen *et al.*

[2011] used 177 *G. bulloides* shells from the size fraction 300–355 μm from core-top samples (box cores NIOBP902–907 collected in close proximity to the NIOBP905 station (Figure 2a). Quoted analytical reproducibility of a carbonate laboratory standard was 0.15‰, which is comparable with our method. The combined data set from *Ganssen et al.* [2011] and our study provide a unique opportunity to study differences in *G. bulloides* geochemistry between eutrophic upwelling (Western Arabian Sea) and oligotrophic (Eastern Arabian Sea) surface waters.

3. Results

3.1. Genetic Variability of *Globigerina bulloides*

A total of 20 specimens of *G. bulloides* Type Ia (Figures 1a and 1b) were identified along the cruise tracks of cruises M74/1b and CD148 in the Arabian Sea (Figure 2a). This genotype is identical to the warm water genotype previously identified in the Coral Sea [Darling and Wade, 2008] (Figure 1a). In the Arabian Sea, Type Ia was found in both upwelling and also in warm open ocean, nonupwelling waters. An additional *G. bulloides* genotype, Type IIb (Figures 1a and 1b), was also identified during cruise M74/1b within a region of late summer upwelling at stations M74/1b-944, 945, and 947 (Figure 2a). This newly recognized genotype was associated with the cooler waters and was not identified in more offshore waters outside the upwelling, though amplification success was low ($n = 7$). The Type IIb genotype falls within the cool water ecological complex, clustering with the Atlantic and Southern Ocean cool water *G. bulloides* genotypes (Figure 1a).

Morphologically, there is little evidence to distinguish the warm Type Ia and the cool Type IIb genotype in the Arabian Sea. Hence, the question arises whether the shells of these two modern genotypes of *G. bulloides* discovered in the surface waters show any distinctive geochemical signatures. The method we used for extracting foraminiferal DNA destroyed the shells and consequently did not allow simultaneous geochemical and genetic studies from the individual foraminifera. We therefore used plankton net samples collected during the same cruise and also core-top samples collected during different cruise but in the same study area to assess whether the geochemical signals of *G. bulloides* display any bimodality, which can be attributed to the two genotypes in this region.

3.2. Mg/Ca Variability of *Globigerina bulloides* From Plankton Assemblages

Seventy-five shells of *G. bulloides* were extracted from plankton net station M74/1b-949 (Figure 2a). Only the top water column sample (i.e., from 0 to 20 m) was used for Mg/Ca analyses where water temperature around $27.3 \pm 0.1^\circ\text{C}$ (1 SD) and salinity near 35.95 ± 0.05 psu (1 SD) prevailed (Figure 2b). The geochemical signals of *G. bulloides* from this assemblage should therefore primarily reflect biological influences (i.e., vital effects), since there was little variation in temperature, salinity, or other environmental factors. Yet Mg/Ca values show significant variability ranging from a minimum value of 3.78 ± 0.36 mmol/mol (1 SE) to a maximum value of 12.5 ± 0.8 mmol/mol (1 SE) (Figure 3a). The distribution of Mg/Ca values also show bimodality (two modes), with low values of clustering around ~ 5.5 mmol/mol and higher values around ~ 9.5 mmol/mol (Figure 3a). Shapiro-Wilk normality test performed on raw Mg/Ca data returned $W = 0.9598$ and $p = 0.0201$, suggesting strong nonnormality in data distribution [Shapiro and Wilk, 1965]. This nonnormality is clearly visible in distribution of calcification temperatures derived for our Mg/Ca values using the *Elderfield and Ganssen* [2000] calibration (Figure 3b). The two peaks (or modes) in temperature distribution are centred at 24.3°C and 28°C , revealing a large temperature difference ($\sim 4^\circ\text{C}$) between the two populations. Despite this bimodality the average calcification temperatures derived from the *Elderfield and Ganssen* [2000] calibration is within 1°C from observed water temperature (i.e., $27.3 \pm 0.1^\circ\text{C}$ measured versus $26.2 \pm 0.3^\circ\text{C}$ calculated). This accuracy, however, masks the true bimodal origin of the geochemical signals in living *G. bulloides* assemblages in the Arabian Sea which likely reflect two distinct populations.

3.3. Oxygen Isotopes Variability of *Globigerina bulloides* From Sediment Assemblages

We use core-top samples from sediment cores SK17 and NIOBP905 (Figure 2a) to study the distribution of $\delta^{18}\text{O}$ values within sediment assemblages of *G. bulloides*. Site SK17 is located in a region of the Eastern Arabian Sea (EAS) characterized by stratified oligotrophic surface waters with temperatures above 26°C throughout most of the year [Boyer et al., 2013]. The distribution of $\delta^{18}\text{O}$ values of *G. bulloides* shells from the EAS (site SK17) is relatively narrow and closely resembles a normal distribution (Figure 4a), which would be consistent with the assumed presence of single genotype. Shapiro-Wilk normality test for $\delta^{18}\text{O}$ values returned $W = 0.9808$ and

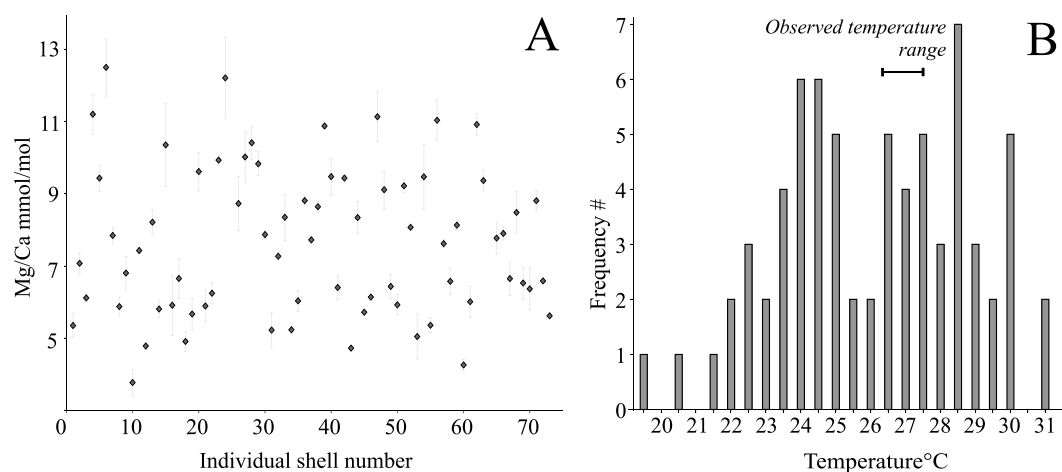


Figure 3. Distribution of Mg/Ca values of *G. bulloides* plankton assemblage in the Arabian Sea. (a) Individual shell Mg/Ca values of *G. bulloides* from plankton net sample 949 in the Western Arabian Sea. The error bars represent the standard error of the means (\pm) derived from multiple measurements of individual shells. (b) Distribution of calcification temperatures derived from *G. bulloides* Mg/Ca values, assuming its temperature dependence as in Elderfield and Ganssen [2000]. The small horizontal bar represents the observed seawater temperature within the top 20 m of the water column during sample collection.

$p = 0.5338$, confirming normal distribution of the data. The mean $\delta^{18}\text{O}$ value is -2.46‰ with a standard deviation of 0.4‰ and range of 1.94‰ . Using these values, we have reconstructed the temperature range and habitat depths for these *G. bulloides* shells using modern day Arabian Sea temperature and salinity values [Boyer et al., 2013] together with empirical calibrations from Bemis et al. [1998] for temperature and for salinity Delaygue et al. [2001] (Figure 2a). The habitat reconstructions indicate that the majority of individuals lived between 50 and 90 m depth (Figure 4a) with a total range between 0 and 150 m. This depth range fits well with the known ecology of *G. bulloides* in the Arabian Sea [Peeters et al., 2002].

In contrast to the EAS, pronounced seasonal changes between nonupwelling and upwelling conditions occur off the coasts of Oman and Somalia in the Western Arabian Sea (WAS) [Sheppard et al., 1992]. The prevailing south-westerly summer-monsoon winds induce upwelling and drive cold, nutrient-rich waters to the surface. Surface water temperatures during peak upwelling conditions may fall as low as $14\text{--}20^\circ\text{C}$ [Brock et al., 1992]. The sediment population of *G. bulloides* shells from site NIOP905 in the WAS has a much wider $\delta^{18}\text{O}$ range of approximately 4.5‰ in comparison to EAS site SK17 (Figures 4a and 4b). The distribution is negatively skewed and has two peaks/modes: a major one at approximately -1‰ and a satellite peak at -2.2‰ . The distribution of $\delta^{18}\text{O}$ values in core NIOP905 also failed the Shapiro-Wilk normality test ($W = 0.9776$ and $p = 0.0062$), indicating strong nonnormal distribution. Using $\delta^{18}\text{O}$ values, we have reconstructed the temperature range and depth habitats of the WAS core top *G. bulloides* in a similar manner as in the EAS. The reconstructed temperature range using calibrations from Bemis et al. [1998] and Delaygue et al. [2001] is between 10 and 28°C (Figure 4b). This temperature range implies that *G. bulloides* shells were formed at ~ 700 m depth during peak upwelling (August) as well as at the sea surface during nonupwelling conditions (Figure 4b). Observations in the modern ocean, however, show that *G. bulloides* inhabits the surface water down to approximately 200 m depth, with a major peak in abundance at the chlorophyll maximum, which varies during the year from 0 to 100 m [Banse, 1987; Schiebel et al., 1997; Peeters et al., 2002]. This calculation, however, is subject to calibration selection, and the exact calcification temperatures are difficult to reconstruct (see more in section 4).

4. Discussion

Our results indicate a bimodal distribution for both the $\delta^{18}\text{O}$ and Mg/Ca values in the core top and living assemblages of *G. bulloides* in the Arabian Sea. This bimodality cannot be explained solely by environmental signals, which show little covariance with the observed distribution and amplitude of $\delta^{18}\text{O}$ and Mg/Ca values. Interpretation of the $\delta^{18}\text{O}$ values of individual shells from core-top samples is complicated by the fact that

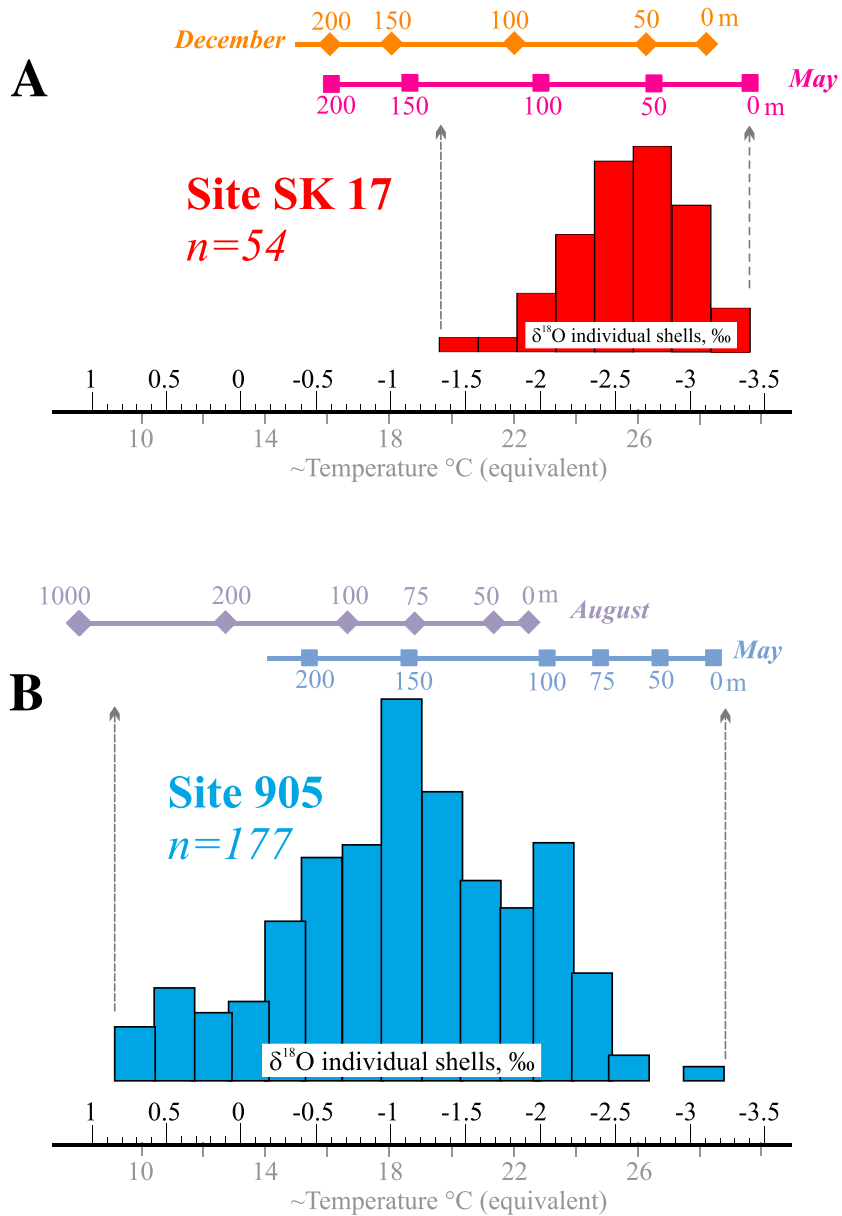


Figure 4. Distribution of oxygen stable isotope values of *G. bulloides* sediment assemblages in the Arabian Sea. (a) Distribution histogram of the $\delta^{18}\text{O}$ values of 54 individual *G. bulloides* shells from the Eastern Arabian Sea (site SK17, see Figure 2a). Above are the predicted $\delta^{18}\text{O}$ values of *G. bulloides* calcite at different depths for the coldest (December, diamonds) and warmest (May, squares) months. The calculation is based on empirical calibrations from Bemis *et al.* [1998] (temperature) and Delaygue *et al.* [2001] (salinity) using the temperature and salinity data from World Ocean Atlas [Boyer *et al.*, 2013] for the Eastern Arabian Sea. Calcite $\delta^{18}\text{O}$ values are assumed to be in equilibrium with seawater. Note that relatively narrow distribution corresponds to the 0–200 m water interval and agrees well with the known *G. bulloides* ecology. (b) Distribution histogram of the $\delta^{18}\text{O}$ values of 177 individual *G. bulloides* shells from a series of core tops in the Western Arabian Sea [Ganssen *et al.*, 2011]. Note the wide range of $\delta^{18}\text{O}$ values, which is equivalent to approximately 20°C. Above are the predicted $\delta^{18}\text{O}$ values of *G. bulloides* calcite at different depths for the coldest (August, diamonds) and warmest (May, squares) months for the Western Arabian Sea. The calculation is based on empirical calibrations from Bemis *et al.* [1998] (temperature) and Delaygue *et al.* [2001] (salinity) data from the World Ocean Atlas [Boyer *et al.*, 2013]. Calcite $\delta^{18}\text{O}$ values are assumed to be in equilibrium with seawater. Note that the coldest $\delta^{18}\text{O}$ values for the Western Arabian Sea in August predict an improbable calcification depth of 800 m when only environmental signals are taken into consideration.

they represent an average of the environmental conditions prevailing over the decades of time taken to accumulate on the seafloor. For example, *Ganssen et al.* [2011], using a different set of calibrations for $\delta^{18}\text{O}$ values, were able to explain all the variance by environmental parameters. In addition, $\delta^{18}\text{O}$ values of fossil shells may be affected by postdepositional alteration, which is known to occur in sediment assemblages [*Elderfield and Ganssen, 2000; Rosenthal and Lohmann, 2002*]. On the other hand, Mg/Ca values of shells from living specimens only reflect the environmental signals in the water column occurring immediately prior to sample collection by the biological activity of the foraminiferal cell. The plankton sample used in this study was collected from a well-developed mixed layer with minimal seawater temperature and salinity variations (Figure 2b). Although there are no direct measurements of seawater carbon system parameters (i.e., pH and CO_3^{2-}), it is unlikely that the upper 20 m of the mixed layer would have had variations significant enough to cause detectable changes in Mg/Ca values, taking into consideration their limited effect on foraminiferal Mg/Ca values [*Russell et al., 2004*]. Mg/Ca measurements in shells from the plankton sample were also obtained from the last three final chambers, limiting vertical water migration, or advection to only 3 to 6 days of the foraminiferal life span. Therefore, assuming little variation in seawater parameters, the observed range and bimodal distribution of Mg/Ca values must be related to biological control of Mg incorporation into *G. bulloides* calcite.

The biological effect on trace metal incorporation, or so-called *vital effect*, is used to describe various processes potentially related to foraminiferal biology and biomineralization. Little is known about the processes controlling Mg incorporation into foraminiferal calcite [*Bentov and Erez, 2006*], and therefore, the vital effect has been used to describe any influence on Mg/Ca, which cannot be directly explained by physical seawater parameters. For example, the symbiont-bearing foraminifera have a distinct microenvironment around their shells. The carbonate ion concentrations and pH of this microenvironment significantly differ from the surrounding seawater, which can be related to the photosynthetic and respiration activity of their algal symbionts. This alteration of foraminiferal microenvironments has been used to explain differences in Mg/Ca values between planktonic foraminifera and species specific Mg/Ca temperature sensitivity [*Eggins et al., 2004; Barker et al., 2005*]. Broadly, however, vital effect can be subdivided into two distinct groups, which are relevant for this work. The first group comprises factors related to genetically programmed processes, which are unique for each species or genotype. Examples of these processes include mechanisms controlling Mg concentration in the calcifying fluid or harboring a specific type of symbiont in its cytoplasm. The second group includes ontogenetic changes in the processes described above. In other words, these are differences in Mg incorporation between juvenile and adult foraminifera. Shell size effect on Mg/Ca values of planktonic foraminifera is a well-known example of such vital effect [*Elderfield et al., 2002; Anand and Elderfield, 2005*].

Both genetic and ontogenetic vital effects could be potentially used to explain the observed bimodal distribution of Mg/Ca values in the plankton assemblage from the Arabian Sea. The size fraction ($>150\ \mu\text{m}$) used to select the *G. bulloides* shells for Mg/Ca analyses is large enough to include individuals from significantly different ontogenetic stages. A culturing experiment by *Spero and Lea* [1996] showed significant differences in $\delta^{18}\text{O}$ signals between *G. bulloides* with 11 and 13 chambers and also differences in $\delta^{18}\text{O}$ temperature dependences between these ontogenetic stages. To explore the potential effect of shell size on the observed Mg/Ca distribution, we used plankton net sample 949 and compared shell sizes with individual Mg/Ca values in the plankton assemblage of *G. bulloides* (Figure 5). We found no relationship between average shell Mg/Ca and its size. Moreover, the two populations of Mg/Ca values can be traced across all size fractions (dashed lines in Figure 5a). However, the average shell Mg/Ca in our study was obtained by combining Mg/Ca signals from the last three/four chambers of *G. bulloides*, and therefore, it is hypothetically possible that contributions of individual chambers to shell average Mg/Ca value could vary across the plankton assemblage producing the observed bimodal Mg/Ca distribution. To exclude this possibility we adopted the *Spero and Lea* [1996] approach to numbering the ontogenetic stages in *G. bulloides* growth. The chamber number was calculated based on a growth curve of *G. bulloides* in culturing experiments [*Spero and Lea, 1996*]. We measured total shell size (i.e., with final chamber) and shell size without the final chamber to estimate the comparability between the growth curve of *Spero and Lea* [1996] and the shells from our plankton sample. The *Spero and Lea* [1996] growth data fit well with our morphometric data from the *G. bulloides* plankton assemblage in the Western Arabian Sea (Figure 5b). The majority of the shells in our plankton sample represent two stages of *G. bulloides* ontogeny. Shells with 11 chambers account for ~45% of the total assemblage, whereas shells with 12 chambers represent ~50% (Figure 5c). Mg/Ca values of individual chambers show no dependency on

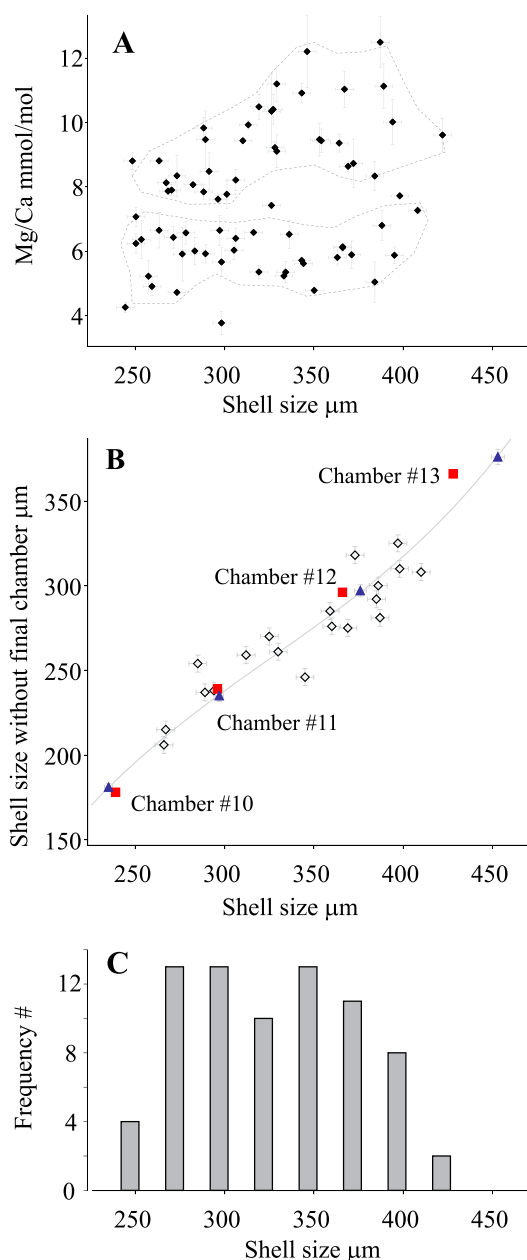


Figure 5. Shell size variation in the planktonic assemblage of *G. bulloides* in the Western Arabian Sea. (a) Crossplot of shell sizes and Mg/Ca values of individual *G. bulloides* shells from plankton net sample 949 in the Western Arabian Sea. Note the lack of any relationship between Mg/Ca values and shell size and also the two potential subpopulations, outlined by thin dashed lines. (b) Distribution histogram of *G. bulloides* shell sizes from plankton net sample 949 in the Western Arabian Sea. (c) Ontogeny of *G. bulloides* as a function of shell size. The x axis represents shell size including the shell's final chamber, and the y axis represents shell size excluding the final chamber. The grey diamonds are data produced in this study for the shells from plankton net sample 949. The squares (red) and triangles (black) are data from the *Spero and Lea* [1996] culturing experiments for 17°C and 21°C seawater temperatures respectively. The solid grey line is a fit through the *Spero and Lea* [1996] data from the 21°C experiment. Note the good agreement between the growth extension of the *Spero and Lea* [1996] culturing experiments and the shells from the Western Arabian Sea plankton assemblage. All three plots in Figures 5a–5c are on the same horizontal axis.

chamber number nor shell size (Figure 6). We found no correlation between the presence of spines or coiling direction of *G. bulloides* shells and their Mg/Ca composition. Crust layers were also not present on any *G. bulloides* shells, which is consistent with their plankton origin. Hence, *G. bulloides* ontogeny does not affect Mg incorporation into shell calcite in our study. This leaves a genetic vital effect as the only potential factor responsible for the observed bimodal distribution of Mg/Ca values in the *G. bulloides* plankton population from the Western Arabian Sea.

Biological vital effects or species/genotype-specific fractionation of trace metals and oxygen stable isotopes has been known since pioneering work was carried out on foraminiferal geochemistry. The best example of this genotype-specific fractionation is the difference in geochemistry between white and pink genotypes of *Globigerinoides ruber* [Darling et al., 1997; Aurahs et al., 2011]. Both Mg/Ca and $\delta^{18}\text{O}$ of these genotypes/species have significantly different temperature dependences and often found to be offset from each other by constant values [Deuser et al., 1981; Williams et al., 1981; Anand et al., 2003; Richey et al., 2012]. Recent estimates of evolution rates in the *Globigerinoides* lineage put the evolutionary split between white and pink *G. ruber* between 4.2 Ma and 8.4 Ma [Aurahs et al., 2011]. This time interval therefore was sufficient to develop a different genotype/species-specific vital effect. The rate of evolution through time in the *Globigerina bulloides* cluster is unknown, since all divergences are morphologically cryptic. However, the genetic distance (i.e., the number of mutations) between warm (Type I) and cool (Type II) genotypes of *G. bulloides* is comparable if not greater than the distance between the pink and white Type I genotypes/species of *Globigerinoides ruber* in the spinose

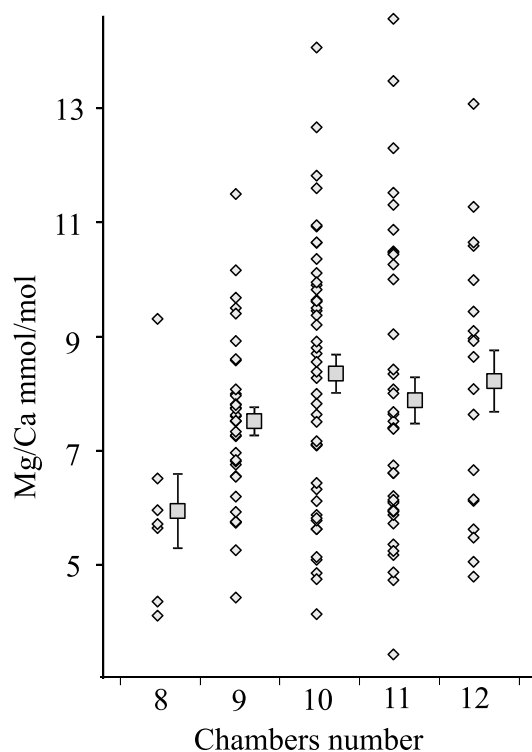


Figure 6. Mg/Ca values of individual chambers of each studied *G. bulloides* shells from plankton assemblage from Western Arabian Sea (grey diamonds). Squares are averaged Mg/Ca values for each chamber, and error bars are standard error of the mean (\pm). Chamber numeration follows the approach adapted in Spero and Lea [1996] culturing experiments. Note that the averaged Mg/Ca values of chambers 9–12 are identical within error bars suggesting lack of Mg/Ca changes during these ontogenetic stages of *G. bulloides*.

planktonic foraminiferal lineage [Sears *et al.*, 2012]. As mentioned above, species delineation studies also indicate that the warm and cool lineage division is well above species level, since species status is supported for several of the *Globigerina bulloides* genotypes in both complexes [Andre *et al.*, 2014]. Considering the strong evidence for genetically controlled bimodality in Mg/Ca values in the *G. bulloides* population from the Western Arabian Sea, we suggest that this bimodality reflects the presence of both warm lineage Type Ia and cool lineage Type II genotypes in this area (Figures 1 and 2). These genotypes/species have distinct and genotype-specific control on Mg incorporation into shell calcite, which produce approximately 3 mmol/mol difference at seawater temperatures of 27°C. This difference is equivalent to $\sim 4^\circ\text{C}$ using current temperature calibrations [Elderfield and Ganssen, 2000; McConnell and Thunell, 2005]. Such a large discrepancy therefore should be considered in paleoclimate reconstructions when using *G. bulloides* shells from areas inhabited by both warm (Type I) and cool (Type II) genotypes. Evidence of bimodality in the sediment population of *G. bulloides* in the Western Arabian Sea and particularly the large range of values may also point to similar genotype-specific $\delta^{18}\text{O}$ fractionation of warm (Type I) and cool (Type II) genotypes (Figure 4). However, potential postdepositional alteration of $\delta^{18}\text{O}$ signals in sediment populations poses greater uncertainty in linking bimodality of *G. bulloides* $\delta^{18}\text{O}$ values to genotype-specific $\delta^{18}\text{O}$ fractionation, and further works are required to clarify this.

G. bulloides is one of the most commonly used planktonic foraminifera for palaeoclimate reconstructions in transitional and upwelling regions [Elderfield and Ganssen, 2000]. The co-occurrence of geochemical signals from both warm water (Type I) and cool water (Type II) genotypes in deep-sea sediments causes difficulties in using this species complex for paleoclimate reconstructions, since they cannot currently be separated by using shell morphology. In addition, published work on *G. bulloides* geochemistry from frontal zones and upwelling areas provides mounting evidence on disparities between seawater properties and *G. bulloides* geochemistry [Oba and Murayama, 2004; Rogerson *et al.*, 2004; Anand *et al.*, 2008; Peck *et al.*, 2008; Martinez-Boti *et al.*, 2011; van Raden *et al.*, 2011; Jonkers *et al.*, 2013]. For example, significant differences in Mg/Ca signals of summer and winter populations of *G. bulloides* have been reported in recent studies of plankton net studies in the Western Arabian Sea [Friedrich *et al.*, 2012]. Friedrich *et al.* [2012] found that Mg/Ca values of *G. bulloides* from cold/upwelling samples agree well with predicted values using the temperature equation of Elderfield and Ganssen [2000]. Sea surface temperature estimates using summer Mg/Ca values, however, were 4–5°C lower than the predicted values by the same thermometer. The other possible example of genotype-specific difference in *G. bulloides* geochemistry is a reported ~ 0.9 – 1.1‰ offset between $\delta^{18}\text{O}$ values of two *G. bulloides* morphotypes coexisting in the Southern California Bight [Sautter and Thunell, 1991; Spero and Lea, 1996; Osborne *et al.*, 2016]. Inconsistencies in *G. bulloides* geochemistry in both of these examples can now be readily explained by using results of our work and genotype-specific Mg and $\delta^{18}\text{O}$ fractionation of warm (Type I) and cool (Type II) *G. bulloides* genotypes. These also provide strong testimony that disparities in *G. bulloides* geochemistry should not be ignored in proxy calibrations and palaeoclimate studies.

5. Conclusions

Results of this work on the genetic variability of *G. bulloides* in the Arabian Sea provide an important contribution toward the growing field of foraminiferal genetic diversity and their complex ecological preferences. Together with geochemical studies and examples from proxy records, our results strongly suggest that the well-accepted approach of using *G. bulloides* morphospecies may potentially result in biased interpretation of past climates in frontal or upwelling ocean regions. We propose that when interpreting palaeorecords from these regions, possible genotype-specific bias in foraminiferal geochemistry should be taken into account. We also suggest that detailed studies of ecology and biodiversity of planktonic foraminifera should be carried out in regions with complex oceanography to assure accuracy of palaeorecords from these locations.

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