BRAZILIAN JOURNAL OF OCEANOGRAPHY, 59(4):327-338, 2011

RELATIONSHIP BETWEEN ISOTOPIC COMPOSITION (Δ^{18}O AND Δ^{13}C) AND PLAKTONIC FORAMINIFERA TEST SIZE IN CORE TOPS FROM THE BRAZILIAN CONTINENTAL MARGIN

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ABSTRACT

Stable oxygen (δ^{18}O) and carbon (δ^{13}C) isotopic signature registered in fossil planktonic foraminifera tests are widely used to reconstruct ancient oceanographic conditions. Test size is a major source of stable isotope variability in planktonic foraminifera found in sediment samples and thus can compromise paleoceanographic interpretations. Test size/stable isotope (δ^{18}O and δ^{13}C) relationships were evaluated in two planktonic foraminifer species (*Globigerinoides ruber* (white) and *Globorotalia truncatulinoides* (right)) in two core tops from the Brazilian Continental Margin. δ^{18}O measurements were used to predict the depth of calcification of each test size fraction. δ^{13}C offsets for each test size fraction were then estimated. No systematic δ^{18}O changes with size were observed in *G. ruber* (white) suggesting a similar calcification depth range (c.a. 100 m) during ontogeny. For *G. truncatulinoides* (right) δ^{18}O values increased with size indicating ontogenetic migration along thermocline waters (250-400 m). δ^{13}C measurements and δ^{13}C offsets increased with size for both species reflecting well known physiological induced ontogenetic-related variability. In *G. ruber* (white) the largest test size fractions (300µm and >355µm) more closely reflect $\delta^{13}C_{DIC}$ indicating they are best suited for paleoceanographic studies.

RESUMO

O tamanho de testa dos foraminíferos é uma importante fonte de variabilidade isotópica (δ^{18}O e δ^{13}C) em amostras de sedimento marinho comprometendo as interpretações paloeceanograficas. No presente estudo, avaliou-se a relação entre o sinal isotópico medido em diferentes frações de tamanho de testa das espécies planctônicas, *Globigerinoides ruber* (branca) e *Globorotalia truncatulinoides* (dextral) em amostras de topo de dois testemunhos localizados na Margem Continental Brasileira. Os valores de δ^{18}O foram utilizados para estimar a profundidade de calcificação de cada fração de tamanho. Os desequilíbrios nos valores de δ^{13}C para cada fração de tamanho foram estimados. Os valores de δ^{18}O em *G. ruber* (branca) não apresentaram tendência com o tamanho sugerindo que calcifica dentro de um mesmo intervalo de profundidade (c.a. 100 m) durante a ontogenia. Os valores de δ^{18}O em *G. truncatulinoides* (dextral) apresentaram aumento com o tamanho refletindo a migração ontogênica em águas da termoclina (250-400 m). Os valores e desequilíbrios de δ^{13}C aumentaram com o tamanho nas duas espécies indicando o efeito da variação nas taxas fisiológicas durante a ontogenia. Em *G. ruber* (white) os valores de δ^{13}C dos maiores tamanhos (300µm e >355µm) refletem melhor os valores de $\delta^{13}C_{DIC}$ indicando que são mais apropriados para utilizar nas reconstruções paleoceanograficas.

Descriptors: Planktonic foraminifera, Stable isotopes, Test size.
Descritores: Foraminíferos planctônicos, Isótopos estáveis, Tamanho testas.

INTRODUCTION

During the calcification process, planktonic foraminifera record the stable isotope signature and environmental conditions of seawater of their calcification depth in a predictable way (ROHLING; COOKE, 1999). As a consequence, stable oxygen (δ^{18}O) and carbon (δ^{13}C) isotopes measured in tests of fossil planktonic foraminifera are widely used in paleoceanography as proxies for paleotemperature, ice volume, water masses movements and paleoproductivity (WEFER et al., 1999; ROHLING; COOKE, 1999). However, in some planktonic species measured stable isotope composition often shows offsets from predicted values (RAVELO; FAIRBANKS, 1995).

These offsets are associated with foraminiferal physiological processes such as respiration, calcification and photosynthesis (in symbiont bearing species) (SPERO; LEA, 1993;

ZEEBE et al., 1999). The carbonate ion effect, associated with calcification processes, also induces offsets during calcification of $\delta^{13}C$ and $\delta^{18}O$ (SPERO et al., 1997). Both laboratory and field based (with living and fossil foraminifera) studies have demonstrated that these offsets are higher regarding $\delta^{13}C$ than $\delta^{18}O$ values. $\delta^{18}O$ measurements in tests of recent planktonic foraminifera can thus be used to evaluate $\delta^{13}C$ offsets (ELDERFIELD et al., 2002; RAVELO; FAIRBANKS, 1995).

Test size is a major source of stable isotope intra-specific variability found in sediment samples. Some of the stable isotopic offsets observed in planktonic foraminifera species are related to test size, having important implications when choosing foraminifera test size fractions for paleoceanographic interpretations (OPPO; FAIRBANKS, 1989). The size-related stable isotope variability can be caused by 1) variation in physiological rates during ontogeny (RAVELO; FAIRBANKS, 1995) and 2) variability in calcification depth during the life cycle (e.g., depth migrations along the water column; LOHMANN, 1995), and during different environmental conditions (e.g., different seasons; DEUSER et al., 1981).

The complexity of isotopic fractionation in foraminifera and its relationship with local environmental condition suggests the importance of evaluating size-related stable isotope variability in areas of paleoceanographic interest. Several authors have used stable isotopes measurements in planktonic foraminifera from the Brazilian Continental Margin as proxies for ancient hydrographic conditions (ARZ, 1998, 1999; TOLEDO et al., 2007). However, there are no local calibration studies concerning both size-related stable oxygen and carbon isotopes in planktonic foraminifera.

The most straightforward approach for the evaluation of paleoceanographic proxies is the analysis of core top samples and its relationship with modern hydrographic conditions. The present work explores sieve size-related stable isotope oxygen and carbon variability in two species of planktonic foraminifera, *Globigerinoides ruber* (white) and *Globorotalia truncatulinoides* (right), in two core tops from the Brazilian Continental Margin. Additionally, we estimated the depth of calcification reflected by measured $\delta^{18}O$ values retained in each sieve size fraction and analyzed which test size fraction best reflects seawater carbon stable isotope signature in the previously estimated calcification depth.

MATERIALS AND METHODS

Cores and Oceanographic Context

We measured the oxygen and carbon isotopes of planktonic foraminifera from two piston core tops, KF-F and KF-G (Table 1) recovered from the Brazilian Continental Margin in the southwestern South Atlantic (Fig. 1).

Table 1. Location, depth and recovery of KF-F and KF-G cores.

Core data	KF-F	KF-G
Geographical position	14º44’S, 38º59’W	20º51’S, 39º08’W
Depth	1957 m	2290 m
Recovery	408 cm	460 cm

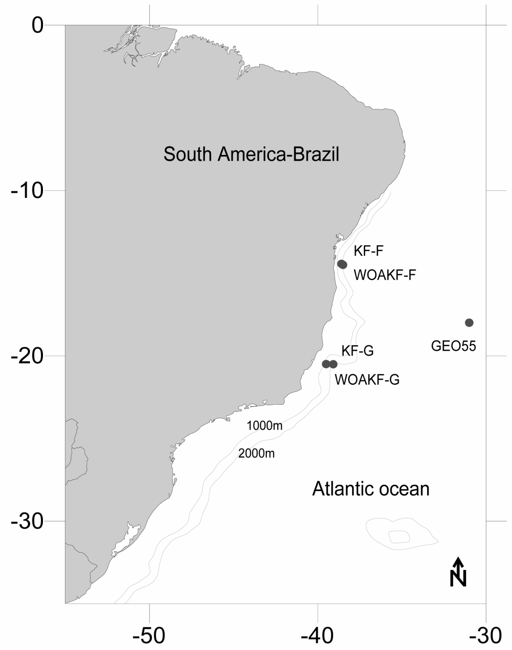

Fig. 1. Study area showing core top locations (KF-F and KF-G), WOA05 data (WOAKF-F and WOAKF-G) and GEOSECS data (GEO55).

The Brazilian southeastern margin is characterized by the stacking of several water masses, including Tropical Water (TW), South Atlantic Central Water (SACW), Antarctic Intermediate Water (AAIW), North Atlantic Deep Water (NADW) and the Antarctic Bottom Water (AABW). The surface waters result from the mixing of three water masses: the TW, a hot (T>18.8ºC) and high-salinity water (S> 36%), the coastal waters and the waters resulting from vertical excursions (upwelling) of the SACW

(GARFIELD, 1990). The SACW is cooler (T<18.8ºC) and less saline (S<36%) (DUARTE; VIANA, 2007).

Chronology and Isotopic Measurements

Chronology was based on the correlation of the isotopic record of benthic foraminifera with SPECMAP chronology of Martinson et al. (1987) and on radiocarbon data using Accelerator Mass Spectrometer (AMS) radiocarbon dating performed on two samples of monospecific planktonic foraminifera (*G. ruber*) from the core top sample of each core (1cm - KF-F and 1.5cm - KF-G). The ^{14}C-AMS dating were performed at NOSAMS- WHOI laboratory facility, and all the ^{14}C ages were corrected for a reservoir effect of 400 yrs and transformed into calendar yrs (BARD, 1993).

Stable isotopes analyses were carried out at Woods Hole Oceanographic Institution, MA, USA using a Finnigan MAT253 mass spectrometer with the automated Kiel device. Isotope values are represented in standard δ notation relative to the Vienna Pee Dee Belemnite (VPDB) for calcite and Vienna Standard-Mean-Water (VSMOW) for sea water. Calibration of the PDB was done via the NBS-19 with an accuracy of ±0.07 for δ^{18}O and ±0.03 for δ^{13}C.

Sampling Procedure

Cores were previously sub-sampled every 5-10cm and samples washed with distilled water over a 63µm mesh size and dried at 50ºC. In order to work with Holocene samples, the three core top samples of each core with lower δ^{18}O values (based on available *Globigerionides ruber* stable oxygen isotopes) were selected: 1cm, 6cm and 11cm from KF-F and 1.5cm, 9cm and 16cm from KF-G.

The samples were separated in the following sieve size fractions: 150-250µm, 250-300µm, 300-355µm and >355µm according to availability of some of the more frequent sieve size fractions used in calibration (ELDERFIED et al., 2002; RAVELO; FAIRBANKS, 1992, 1995) and reconstruction (ARZ et al., 1998, 1999; TOLEDO et al., 2007) studies. The surface dwelling *G. ruber* (white) and the deep dwelling *Globorotalia truncatulinoides* (right) species were identified based on Van Morkoven et al. (1986) and Hemleben et al. (1989). Stable isotope measurements were conducted on between three and six *G. ruber* (white) tests and on between three and four *G. truncatulinoides* (right) tests depending on the size fraction. In KF-F, 4 replicates samples of *G. ruber* (white) were analyzed in the 150-250µm and 250-300 µm size classes allowing evaluation of within size fraction variability. The largest individuals of each size fraction were picked to improve sample comparison. Tests with apparent good preservation (e.g., without fragmentation and color or texture modification) were picked and samples with apparent attached sediments were washed by ultrasonification.

Stable Isotope / Test Size Relationship

The effect of size fraction on each species for both corers was evaluated by a one-way analysis of variance (ANOVA). The Kolmogorov-Smirnov D statistic was used to check for normality, while Cochran's C-test was used to check the assumption of homogeneity of variances. In cases where homogeneity was not achieved, we set the critical level to a value equal to the p-value for variance homogeneity (UNDERWOOD, 1997). All analyses were performed separately for both isotopic values of each species.

Hydrographic Data and Isotopic Composition in the Water Column

Hydrographic data (annual temperature and salinity) along the surface water masses from two stations next to each core top location were extracted from the World Ocean Atlas, 2005 (WOA05) (LOCARNINI et al., 2006; ANTONOV et al., 2006) and are henceforth called WOAKF-F and WOAKF-G for proximity to cores KF-F and KF-G, respectively (Fig. 1). These data was used to identify surface water masses and for estimating oxygen stable isotope values in seawater ($\delta^{18}O_W$) and in equilibrium with seawater ($\delta^{18}O_{EQ}$). Water masses and mixed layer limits were based on Silveira et al. (2000) and Tomczak and Godfrey (1994), respectively.

$\delta^{18}O_W$ values were estimated based on annual salinity WOA05 data and δ^{18}O/salinity equations from Legrand and Schmidt (2006). Tropical Atlantic (eq. 1) and South Atlantic (eq. 2) equations (LEGRAND; SCHMIDT, 2006) were used to estimate $\delta^{18}O_W$ for TW and SACW water masses, respectively.

$$\delta^{18}O_W = S * 0.15 - 4.61 \qquad \text{(eq. 1)}$$

$$\delta^{18}O_W = S * 0.51 - 17.40 \qquad \text{(eq. 2)}$$

where $\delta^{18}O_W$ = δ^{18}O of seawater (VSMOW, %) and S = salinity (%)

Predicted Stable Isotope Values and relationship with the Isotopic Measurements in Foraminifera

Planktonic foraminifera dwell and calcify their carbonate tests at the surface of the water column. Foraminiferal δ^{18}O measurements are assumed to be principally a function of stable isotope signature in seawater ($\delta^{18}O_W$) and temperature. Thus, comparison of $\delta^{18}O$ measurements in core top planktonic tests with predicted values of calcite in equilibrium with modern seawater ($\delta^{18}O_{EQ}$) (obtained from paleotemperature equations) will provide information about the calcification depth of each species (RAVELO; FAIRBANKS, 1992; NIEBLER et al., 1999).

We estimated calcite precipitated in isotopic equilibrium with seawater ($\delta^{18}O_{EQ}$) based on $\delta^{18}O_W$ values, annual temperature (WOA05) and using well known paleotemperature equations (see below). $\delta^{18}O_{EQ}$ (VSMOW) were converted to $\delta^{18}O_{EQ}$ (VPDB) values after HUT (1987) equation.

Different inorganic paleotemperature equations have been proposed for calibration and paleoceanographic purposes (SHACKLETON, 1974; KIM; O´NEIL, 1997). Spero et al. (1997) demonstrated that besides temperature, ambient carbonate ion concentration also influence $\delta^{18}O$ calcification in foraminifera. Mulitza et al. (2003) calibrated a new paleotemperature equation using living surface dwelling foraminifera (including *G. ruber*). This paleotemperature equation takes into consideration surface seawater carbonate ion concentration influence on $\delta^{18}O$ measurements. Hence, comparison of $\delta^{18}O$ measurements in surface dwelling foraminifera (e.g., *G. ruber*) with predicted $\delta^{18}O$ of calcite in equilibrium with seawater ($\delta^{18}O_{EQ}$) based on Mulitza et al. (2003) in a more appropriate estimation of the calcification depth for this species. Thereafter, Mulitza et al. (2003) paleotemperature equation (eq. 3) was used to estimate depth of calcification of *G. ruber* (white). Kim and O'Neil (1997) and Mulitza et al. (2003) paleotemperature equations do not differ significantly at thermocline depth (MULITZA et al., 2003). Thus, in order to allow comparisons with other studies based on paleotemperature inorganic equations, Kim and O'Neil (1997) paleotemperature equation (eq. 4) was used to estimate *G. truncatulinoides* (right) depth of calcification.

$$T = 16.1 - 4.64\,(\delta^{18}O - \delta^{18}O_W) + 0.09\,(\delta^{18}O - \delta^{18}O_W)^2 \quad \text{(eq. 3)}$$

$$T= 14.32 - 4.28\,(\delta^{18}O - \delta^{18}O_W) + 0.07\,(\delta^{18}O - \delta^{18}O_W)^2 \quad \text{(eq. 4)}$$

where T = temperature (ºC), $\delta^{18}O$ = predicted $\delta^{18}O$ of calcite in equilibrium with seawater (VSMOW,%) and $\delta^{18}Ow$= $\delta^{18}O$ of seawater (VSMOW, %).

$\delta^{13}C$ equilibrium fractionation and its temperature dependence are still in debate. While Emrich et al. (1970) and Romanek et al. (1992) proposed temperature-dependent and temperature-independent equilibrium equations, respectively, different calibrating studies use $\delta^{13}C_{DIC}$ to predict seawater values (ELDERFIELD et al., 2002). For evaluation of $\delta^{13}C$ offsets in planktonic foraminifera we choose the third approach. $\delta^{13}C_{DIC}$ values were obtained from GEOSECS (station 55; 18ºS, 31ºW) (BAINDBRIDGE, 1981) close to core locations (Fig. 1).

For both species, $\delta^{18}O$ measurements in foraminiferal tests were used to predict the calcification depth for each test-size fraction, and therefore the $\delta^{13}C$ of dissolved inorganic carbon of seawater ($\delta^{13}C_{DIC}$). The $\delta^{13}C$ values in foraminifera are assumed to reflect the $\delta^{13}C$ values of the seawater of calcification and probably physiological effects. Thus, offsets from predicted values of each test size fraction were calculated subtracting $\delta^{13}C$ measured values from the predicted values at the depth of calcification. Only AMS radiocarbon Holocene-dated foraminifera samples (Table 2) were used to compare to surface water $\delta^{18}O_{EQ}$ and $\delta^{13}C_{DIC}$ values.

Table 2. Accelerator Mass Spectrometer (AMS) Dates and Calendar Ages.

Cores	Sample depth (cm)	AMS date (kyr)	Calendar age (kyr)
KF-F	1	1740	1357
KF-G	1.5	3540	3482

RESULTS

Isotopic Composition of Water Column

Hydrographic data and stable isotopic values in seawater are presented in Table 3. TW and SACW limit was established at 150 m. On an annual basis, temperature mixed layer limit was ca. 50 m. Thermocline waters ranged from the mixed layer limit down to ca. 1000 m. $\delta^{18}O_W$ decreased along the water column reflecting the relatively high evaporation in surface waters. $\delta^{18}O_{EQ}$ increased with depth according to the decrease in temperature. Higher $\delta^{18}O_{EQ}$ values at WOAKF-G with respect to WOAKF-F reflects its southernmost location along the Brazilian Continental Margin. Mixed layer $\delta^{18}O_{EQ}$ values estimated using Mulitza et al. (2003) paleotemperature equation ranged between -2.12% and -1.98% in WOAKF-F and between -1.88% and -1.67% in WOAKF-G (Table 3). Thermocline (depth>50 m) $\delta^{18}O_{EQ}$ values estimated using Kim and O'Neil (1997) equation showed minima of -1.39% and -1.12% values in WOAKF-F and WOAKF-G, respectively. Mixed layer $\delta^{13}C_{DIC}$ value was 1.9% and decreased with depth (Table 3), mirroring nutrient water profile.

Table 3. Surface water masses (Tropical Water (TW) and South Atlantic central waters (SACW)), temperature (ºC), salinity (‰), $\delta^{18}O_W$ (‰), $\delta^{18}O_{EQ}$ (‰) estimated from Kim and O`Neil (1997) (KO) and Mulitza et al. (2003) (M) paleotemperature equations and $\delta^{13}C_{DIC}$ (‰).

Oxygen isotopes												Carbon isotopes	
		WOAKF-F					WOAKF-G						
Water depth	Water mass	Temperature	Salinity	$\delta^{18}O_W$	$\delta^{18}O_{EQ\ (KO)}$	$\delta^{18}O_{EQ\ (M)}$	Temperature	Salinity	$\delta^{18}O_W$	$\delta^{18}O_{EQ\ (KO)}$	$\delta^{18}O_{EQ\ (M)}$	Water depth	$\delta^{13}C_{DIC}$
0	TW	26.8	37.0	0.95	-1.53	-2.12	25.6	36.9	0.92	-1.31	-1.88	3	1.9
10	TW	26.8	37.1	0.95	-1.51	-2.10	25.6	36.9	0.92	-1.31	-1.88	5	
20	TW	26.8	37.1	0.96	-1.50	-2.08	25.6	36.9	0.92	-1.30	-1.87		
30	TW	26.7	37.2	0.96	-1.48	-2.06	25.5	36.9	0.93	-1.27	-1.84	28	
50	TW	26.3	37.2	0.97	-1.39	-1.98	24.7	36.9	0.92	-1.12	-1.67	48	2
75	TW	25.4	37.2	0.97	-1.22	-1.79	23.7	36.8	0.91	-0.92	-1.47	83	
100	TW	24.3	37.2	0.96	-1.00	-1.56	22.2	36.6	0.88	-0.65	-1.17		
125	TW	22.9	37.0	0.94	-0.74	-1.27	20.5	36.4	0.85	-0.33	-0.83	128	
150	SACW	21.2	36.8	1.35	0.02	-0.48	19.0	36.2	1.04	0.16	-0.31	176	
200	SACW	17.7	36.1	1.00	0.41	-0.04	16.5	35.7	0.80	0.47	0.04		
250	SACW	15.2	35.6	0.76	0.71	0.30	14.9	35.5	0.72	0.73	0.32	265	1.4
300	SACW	13.4	35.3	0.59	0.94	0.55	13.8	35.3	0.61	0.87	0.48	338	
400	SACW	10.7	34.9	0.40	1.34	0.99	11.7	35.1	0.48	1.20	0.84	436	1
500	SACW	8.2	34.6	0.27	1.78	1.46	9.5	34.8	0.32	1.53	1.20	537	

Foraminifera oxygen stable isotopes

All stable isotope results are presented in Table 4. Maximum $\delta^{18}O$ variability within a same *G. ruber* (white) size fraction (250-300µm) was 0.72%. There were no systematic changes in $\delta^{18}O$ with size for *G. ruber* (white) (Figure 2a, ANOVA; $F_{(3,\ 38)} = 0.895$, $p > 0.05$). Systematic increment in $\delta^{18}O$ with size for *G. ruber* (white) (Fig. 2a, ANOVA; $F_{(3,\ 38)} = 0.895$, $p > 0.05$). Systematic increment in $\delta^{18}O$ with size was evident for *G. truncatulinoides* (Fig. 2a, ANOVA; $F_{(3,\ 20)} = 3.686$, $p < 0.05$).

Comparison of *G. ruber* (white) $\delta^{18}O$ measurements in 1cm (KF-F) and 1.5cm (KF-G) with predicted $\delta^{18}O_{EQ}$ values for surface water above each core top location using Mulitza et al. (2003) (M) equation indicates a depth of calcification of ca. 100 m (Fig. 3a, b). Depth of calcification for *G. truncatulinoides* (right) using Kim and O'Neil (1997) paleotemperature equation (KO) was ca. 250 m for the smallest size fraction (150-250µm) and between 250 m (KF-G) and 400 m (KF-F) for the largest size fraction (>355µm) (Fig. 3a, b).

Foraminifera Carbon Stable Isotopes

Maximum *G. ruber* (white) $\delta^{13}C$ variability within a size fraction (KF-F; 150-250µm) was 0.62%. *G. ruber* (white) $\delta^{13}C$ measurements increased with size (Fig. 2b, ANOVA; $F_{(3,\ 38)} = 36.36$, $p < 0.01$) with a maximum $\delta^{13}C$ difference of 1.85% between extreme size fractions (KF-F; 1cm). *G. truncatulinoides* (right) $\delta^{13}C$ also increased with size (Fig. 2b, ANOVA; $F_{(3,\ 20)} = 15.73$, $p < 0.01$) with maximum variation of 1.26% between extreme size fractions (KF-F; 6cm). *G. truncatulinoides* (right) show lighter $\delta^{13}C$ values than *G. ruber* (white) (Fig. 2b).

Table 4. $\delta^{18}O$ (‰) and $\delta^{13}C$ (‰) values registered in *G. ruber* (white) and *G. truncatulinoides* (right).

		G. ruber (white)			*G. truncatulinoides* (right)		
Core/sample depth	Test size fraction	No. tests	$\delta^{18}O$	$\delta^{13}C$	No. tests	$\delta^{18}O$	$\delta^{13}C$
KFF							
1cm	150-250	6	-1.35	0.59	4	0.58	0.75
	150-250	6	-1.39	0.34			
	150-250	6	-1.60	0.66			
	150-250	6	-1.65	0.61			
	250-300	4	-0.93	1.24	3	0.65	0.98
	250-300	4	-1.65	1.74			
	250-300	4	-1.57	1.21			
	250-300	4	-1.60	1.27			
	300-355	3	-1.54	1.56	3	1.19	1.32
	>355	3	-1.51	2.39	3	1.17	1.41
6cm	150-250	6	-1.33	1.21	4	-0.02	0.12
	150-250	6	-1.61	0.59			
	150-250	6	-1.35	0.88			
	150-250	6	-1.41	1.05			
	250-300	4	-1.06	1.70	3	0.95	1.02
	250-300	4	-1.29	1.22			
	250-300	4	-1.50	1.71			
	250-300	4	-1.56	1.22			
	300-355	3	-1.43	1.23	3	0.78	1.36
	>355	3	-1.29	1.69	3	0.77	1.38
11cm	150-250	6	-1.18	0.91	4	0.02	0.43
	150-250	6	-1.35	1.23			
	150-250	6	-1.32	0.80			
	150-250	6	-0.95	0.94			
	250-300	4	-1.31	1.43	3	0.32	0.73
	250-300	4	-1.00	1.34			
	250-300	4	-0.88	1.40			
	250-300	4	-1.49	1.32			
	300-355	3	-1.37	1.92	3	0.35	1.21
	>355	3	-1.18	2.08	3	1.24	1.45
KFG							
1.5cm	150-250	6	-1.24	1.00	3	0.57	1.09
	250-300	3	-1.19	1.31	3	0.66	1.01
	300-355	3	-1.04	1.63	3	0.93	1.62
	>355	3	-0.77	1.74	3	0.60	1.51
9cm	150-250	6	-0.99	1.08	3	-0.01	0.52
	250-300	3	-0.84	1.61	3	0.91	1.27
	300-355	3	-1.22	1.65	3	0.61	1.40
	>355	3	-0.96	2.09	3	0.60	1.46
16cm	150-250	6	-0.90	1.00	3	0.76	1.09
	250-300	3	-0.86	1.08	3	0.49	0.95
	300-355	3	-1.23	1.33	3	0.90	1.27
	>355	3	-0.97	1.91	3	0.61	1.63

Offsets from predicted values increased with size fractions for both species (Fig. 4a, b). For *G. ruber* (white) $\delta^{13}C$ measurements in the largest size fractions (300-355µm and >355µm) are closer to $\delta^{13}C_{DIC}$ values in the corresponding depth of calcification (offset ≈ 0) (Fig. 4a). Concerning *G. truncatulinoides* (right), the largest size fractions seem to present lower offsets, although this was not so clear (Fig. 4b).

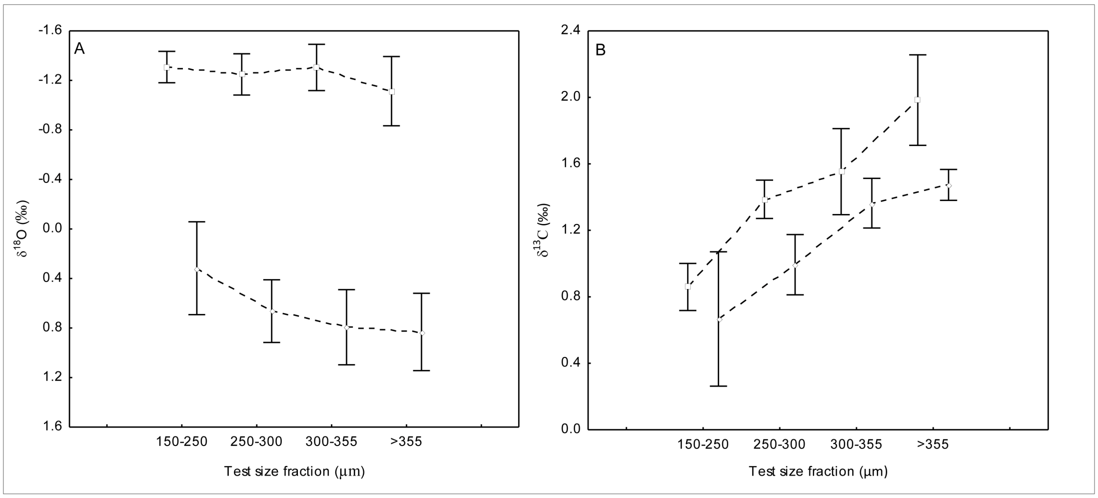

Fig. 2. $\delta^{18}O$ (A) and $\delta^{13}C$ (B) values measured in *G. ruber* (white) (square symbols) and *G. truncatulinoides* (right) (triangle symbols) related to sieve size fractions in all (KF-F and KF-G) samples. Dotted lines join mean values. Vertical bars denote 0.95 confidence intervals.

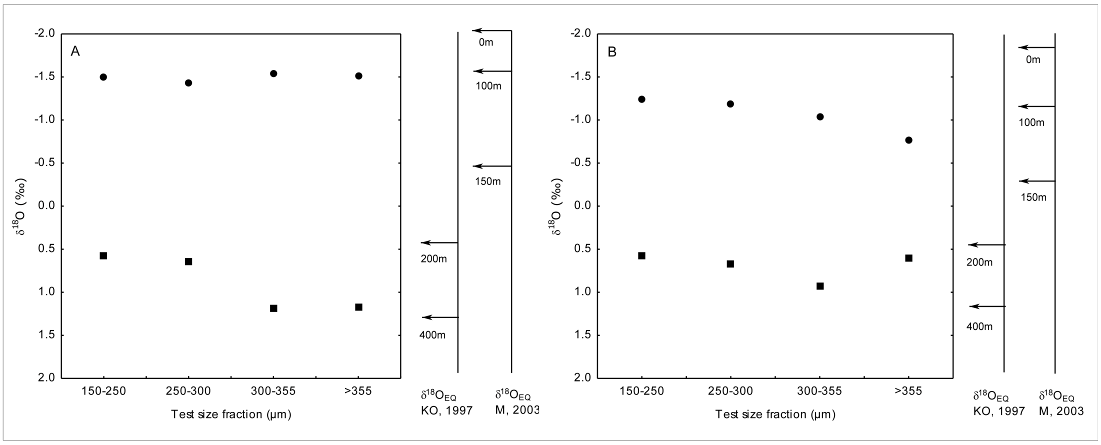

Fig. 3. $\delta^{18}O$ values related to *G. ruber* (white) (open symbols) and *G. truncatulinoides* (right) (close symbols) sieve test size fractions for dated Holocene samples in KF-F (A) and KF-G (B) used to compare with surface water $\delta^{18}O_{EQ}$ values. $\delta^{18}O_{EQ}$ values obtained with Kim and O`Neil (1997) (KO, 1997) and Mulitza et al. (2003) (M, 2003) paleotemperature equations for different surface water depths are shown at each right margin.

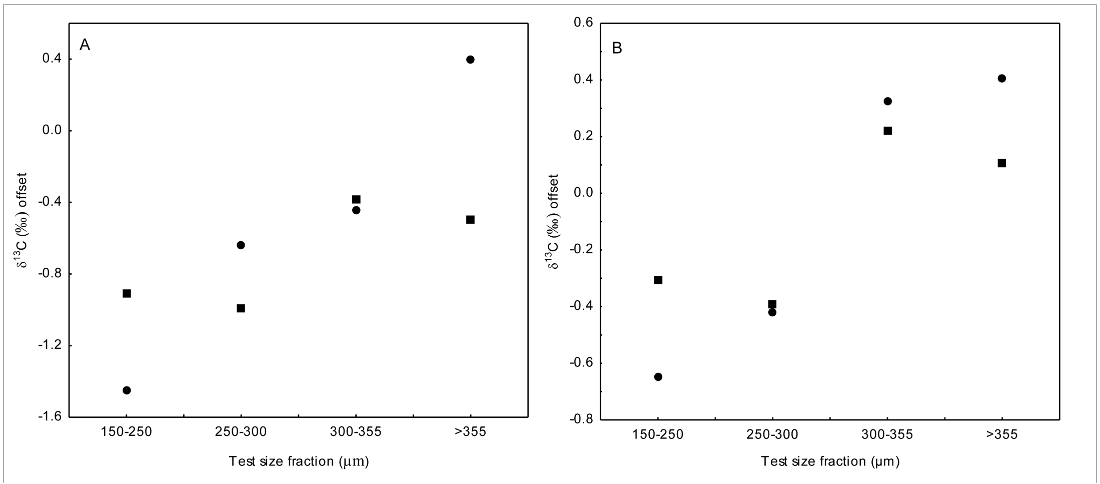

Fig. 3. δ^{13}C offsets ($\delta^{13}C_{forams}$ - $\delta^{13}C_{DIC}$) for *G. ruber* (white) (A) and *G. truncatulinoides* (right) (B) for different sieve test size fractions in KF-F (close symbols) and KF-G (open symbols), dated Holocene samples.

DISCUSSION

Comparison of Core top Samples with Modern Hydrographic Conditions

Even though all samples reflect Holocene surface water conditions, only dated samples were used for comparison with modern hydrological conditions. Comparison of modern hydrographic data and core-top samples may have some methodological limitations concerning time scales. While modern WOA05 data represent mean hydrological conditions from the last decades, core tops used in this study reflect late Holocene conditions, i.e. 1357kyr (KF-F) and 3482kyr (KF-G). However, several authors suggested relatively weak upper water column hydrographic changes during the Holocene from the Brazilian Continental Margin (ARZ, 1998; TOLEDO et al., 2007). Moreover, this approach is commonly used for core top isotopic calibration studies worldwide (RAVELO; FAIRBANKS, 1992, 1995; ELDERFIELD et al., 2002; MULITZA et al., 2003; WAELBROECK et al., 2005).

Stable Isotope (δ^{18}O and δ^{13}C) Variability Within a Single Size Fraction

Several factors can influence δ^{18}O planktonic foraminifera intra-specific variability; variable depth and season of calcification (MULITZA et al., 2003); variable calcification, respiration and photosynthesis rates (WOLF-GLADROW et al., 1999); amount of secondary calcification (HEMLEBEN et al., 1989); different morphotypes (LOWEMARK et al., 2005); dissolution (LOHMANN, 1995) and bioturbation (MULITZA et al., 2003). Regarding δ^{13}C values, the variability in depth and season of calcification as well as variable calcification, respiration and photosynthesis rates (WOLF-GLADROW et al., 1999) can result in important intra-specific variability.

Paleoceanographic interpretations are usually based on a single test size fraction. However, since intra-specific variability is inversely related to the amount of foraminifera tests used per stable isotopic analysis (WAELBROECK et al., 2005), the use of as many tests as possible per isotope analysis (e.g., ca. 20 tests per analysis) (WAELBROECK et al., 2005) has been suggested. This should decrease the stable isotope variability within a single size fraction and identify the average stable isotope signature of a fossil population.

In this study, the maximum intra-specific variability found within replicates of a single size fraction for *G. ruber* (white) using 4-6 tests per analysis was 0.72% for δ^{18}O (KF-F; 250-300µm) and 0.62% for δ^{13}C (KF-F; 150-250µm). These results are in agreement with equatorial Pacific *G. ruber* studies using single test analysis (e.g., 2%, 250-350µm for δ^{18}O and δ^{13}C) (WAELBROECK et al., 2005). During calcification a δ^{18}O increment of 0.25% accounts for a diminution of 1ºC for ambient seawater temperature (ROHLING; COOKE, 1999), whereas during δ^{13}C calcification an increment of 1% accounts for a decrease of 1µmol/kg for ambient phosphate (BROECKER; MAIER-REIMER, 1992). This reflects the importance of increasing the amount of tests used in isotopic analyses when searching for the true average value of each size fraction and/or single sample in the region. The use of a relatively small amount of tests per stable isotope analysis in this study may preclude conclusive stable isotope/test size relationship. However, this was partially solved analyzing replicate samples in some size fractions and several Holocene samples in each core location.

δ^{18}O/test Size Relationship

Studies concerning δ^{18}O/test size relationship in the shallow dwelling *G. ruber* in recent sediment samples along a complete range of test sizes show either a decrease with size (KROON; DARLING, 1995), a slight increase with size (BERGER et al., 1978) or no systematic changes with size (ELDERFIELD et al., 2002; WAELBROECK et al., 2005). The decrease with size was explained as local seasonal environmental factors (irradiance, salinity and/or temperature) affecting both size and stable isotope signature of *G. ruber* (KROON; DARLING, 1995). The increase with size was suggested to reflect the development of adult secondary calcification in cold deeper waters (DUPLESSY et al., 1981). The lack of systematic δ^{18}O/test size variation observed for *G. ruber* (white) suggests that in the study area this species calcify without environmentally-induced test size and stable isotope variations and/or along a similar range of depths during ontogeny.

G. trunatulinoides is a deep dwelling species which undergoes vertical migration during its ontogeny (HEMLEBEN et al., 1989; LOHMANN, 1995). This implies the calcification of an ontogenetic calcite test in shallow, warmer waters during juvenile stages and a secondary calcification crust in deeper, cooler waters during adult stages (HEMLEBEN et al., 1989). Thereafter, *G. truncatulinoides* incorporates a lighter and heavier δ^{18}O signature during primary and secondary calcification, respectively (LOHMANN, 1995; MULITZA et al., 1997). However, its bulk test chemistry is a mixture of two end-member calcite and depends on the relative proportion of each calcite (LOHMANN, 1995). In this work it was not intended to identify calcification crust. Hence, the systematic δ^{18}O increase with size fraction reflect migratory behavior.

Apparent depth of calcification

Several studies have demonstrated the potential of δ^{18}O measurements in core top planktonic foraminifera to reflect species´ calcification depth (NIEBLER et al., 1999; RAVELO; FAIRBANKS, 1992). Plankton studies demonstrated that the symbiont-bearing *G. ruber* (white) is a surface dwelling species associated with the mixed surface layer (KEMLE-VON MUCKE; OBERHANSLI, 1999). In the region, the depth of maximum *G. ruber* density (0-40 m; Sorano, pers. com.) is coincident with the estimated annual mixed layer depth (ca. 50 m). The reflected depth of calcification here estimated (ca. 100 m) agreed with previous reported data from the region (CHIESSI et al., 2007) based on annual temperature and the same paleotemperature equation. The difference between this estimated depth of calcification and the plankton habitat depth may be due to seasonal flux, vertical migration and/or post-depositional effects (MULITZA et al., 2003). However, since seasonal flux in this species seems to occur mainly during summer this should be reflected in a sediment mean flux-weighed shift towards lighter than annual mean δ^{18}O values (MULITZA et al., 2003), thus making this explanation improbable. Although dissolution effects are unlikely because of the relatively shallow sampling water depth (ca. 2000 m) with respect to lysocline depth in the modern South Atlantic ocean (ca. 4100 m; FRENZ; HENRICH, 2007), bioturbation may occur in areas of low sedimentation rates such as the study region (3cm/1000kyr, TOLEDO et al., 2005). Nonetheless, differences between living and sediment *G. ruber* (white) δ^{18}O measurements (0.5-1.0%) were also observed in global studies (MULITZA et al., 2003; WAELBROECK et al., 2005) discarding late Holocene samples with possible bioturbation. In this paper, δ^{18}O / sieve size relationship suggests *G. ruber* (white) calcification along a similar depth range during ontogeny, while Mulitza et al. (2003) and Waelbroeck et al. (2005) suggest calcification in deeper waters during ontogenesis or gametogenesis as a probable explanation of this observed isotopic difference. This difference seems to be higher in low latitudes where stratification of the water column is more pronounced and in areas of low surface salinity (WAELBROECK et al., 2005). These authors stated that the relationship between δ^{18}O equilibrium calcite and δ^{18}O from sediment planktonic foraminifera is complex and depends on local hydrography. Therefore, differences between plankton and sediment *G. ruber* δ^{18}O values must be taken into account during paleoceanographic interpretations based on sediment samples (WAELBROECK et al., 2005).

For the deep dwelling *G. truncatulinoides* (right) the depth of calcification estimated agree with Mulitza et al. (1997) for tropical waters and suggests primary calcification below the highly stratified mixed layer waters (ca. 250 m) and adult calcification of a secondary crust in deeper cold waters (ca. 400 m). Moreover, this adult calcification depth coincides with the temperature known to trigger the calcification of the secondary crust (8-10ºC, HEMLEBEN et al., 1989).

δ^{13}C/test Size Relationship and δ^{13}C offsets

The increase δ^{13}C with size observed in *G. ruber* (white) and *G. truncatulinoides* (right) is well known in other regions in both plankton (WILLIAMS et al., 1981; DUPLESSY et al., 1981) and sediment samples (HEMLEBEN et al., 1989; RAVELO; FAIRBANKS, 1995; ELDERFIELD et al., 2002). This increase is commonly explained invoking different rates of calcification, respiration and photosynthesis (in symbiotic species) along foraminiferal ontogeny. These factors influence the chemistry of the microenvironment of foraminifera and hence the $\delta^{13}C$ registered in the tests (ZEEBE et al., 1999; WOLF-GLADROW et al., 1999). For *G. truncatulinoides* (right) this increase opposed the seawater $\delta^{13}C_{DIC}$ gradient (decrease with depth) contradicting the depth of calcification reflected by $\delta^{18}O$ values. The highest $\delta^{13}C$ values observed in *G. ruber* (white) compared with *G. truncatulinoides* in size fractions agree with other studies (WILLIAMS et al. 1981; RAVELO; FAIRBANKS, 1995) and likely reflects calcification of *G. ruber* in shallower waters.

Physiological effects result in size-dependent offsets from predicted $\delta^{13}C$ values, suggesting the need to limit test size fractions used in paleoceanographic studies. Ravelo and Fairbanks (1995) observed *G. ruber* (white) $\delta^{13}C$ offsets varied with regional hydrography although they attributed it to uncertainties in seawater $\delta^{13}C_{DIC}$ values. The use of small amount of tests per analysis in the largest size fractions together with scarce $\delta^{13}C_{DIC}$ values available in the region precludes evaluating differences in $\delta^{13}C$ offsets between core tops. However, it is clear that $\delta^{13}C$ measurements of the largest size fractions (300-355µm and >355µm) of *G. ruber* (white) are closer to predicted $\delta^{13}C_{DIC}$ values from the estimated depths of calcification. This agree with Elderfield et al. (2002) and Ravelo and Fairbanks (1995) and indicates that physiological size-related effects decrease with size and/or that opposed physiological effects cancel each other in the largest test size fractions. Thus, the largest test size fractions of *G. ruber* (white) are more adequate for paleoceanographic interpretations based on $\delta^{13}C$ measurements. For *G. truncatulinoides* (right) $\delta^{13}C$ offsets also vary with test size indicating physiological size-related influence on $\delta^{13}C$ offsets. Elderfield et al. (2002) observed that the largest size fractions of *G. truncatulinoides* were closer to predicted $\delta^{13}C_{DIC}$ values in the depth of calcification. In this work this was not so clear. This suggests that other factors than size-related physiological effects are influencing $\delta^{13}C$ offsets in *G. truncatulinoides*. This line up with Ravelo and Fairbanks (1995) who observed size-related offsets even using a larger amount of *G. truncatulinoides* tests per analysis. They demonstrated that temperature also affects $\delta^{13}C$ offsets in *G. truncatulinoides* (right) showing the complexity in *G. truncatulinoides* $\delta^{13}C$ fractionation.

CONCLUSIONS AND PALEOCEANOGRAPHIC IMPLICATIONS

Despite limitations due to the use of relatively small amount of tests per analysis and limited local $\delta^{13}C_{DIC}$ values, measurements of isotopic composition ($\delta^{18}O$ and $\delta^{13}C$) of sieve test size fractions of two planktonic foraminifera species (*G. ruber* (white) and *G. truncatulinoides* (right)) in core top samples from the Brazilian Continental Margin allowed evaluation of isotopic variability within and between size fractions. Comparison of measured $\delta^{18}O$ and $\delta^{13}C$ values with predicted modern seawater $\delta^{18}O_{EQ}$ and $\delta^{13}C_{DIC}$ values allowed estimation of the calcification depth and of $\delta^{13}C$ offsets, respectively, for each test size fraction for both species.

Within a single size fraction, maximum variability between replicates using 4-6 tests of *G. ruber* (white) per analysis for $\delta^{18}O$ and $\delta^{13}C$ indicates that the use of a larger amount of tests per isotopic analysis will improve accuracy when searching for within-size fraction average population values.

Considering *G. ruber* (white), lack of significant $\delta^{18}O$ differences between sieve size fractions suggests that this species calcify within a similar depth range during ontogeny. The difference between estimated calcification depth (ca. 100 m) and G. *ruber* mixed layer habitat depth (ca. 50 m) must be taken into account for paleoceanographic interpretations. Regarding $\delta^{13}C$ values, a significant increment with sieve size fraction reflected the influence of physiological effects during fractionation and indicates the importance of limiting the sieve size fraction in paleoceanographic studies. The highest sieve size fractions (300-355µm and >355µm) better reflect $\delta^{13}C_{DIC}$ values of surface waters in the region and thus are more suitable for reconstruction of ancient surface water nutrient conditions.

Regarding *G. truncatulinoides* (right), the $\delta^{18}O$ increment with sieve size fraction reflected the vertical migration of this species along thermocline waters with calcification of juvenile tests in shallower waters (ca. 250 m) and adult tests in deeper cooler waters (ca. 400 m). $\delta^{13}C$ measured values also increased with sieve size fraction, contrasting with the vertical seawater $\delta^{13}C_{DIC}$ gradient. Variation in $\delta^{13}C$ offsets of *G. truncatulinoides* (right) suggests that caution must be taken when using $\delta^{13}C$ measured in this species for paleoceanographic interpretations.

ACKNOWLEDGMENTS

This work was supported financially by the Coordenação de Aperfeiçoamento de Pessoal de Nível

Superior (CAPES). We thank Petrobras for providing the samples. The study benefited greatly from the comments of the two anonymous reviewers. We thank Alvar Carranza for his help with the English version of the manuscript.

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(Manuscript received 07 August 2010; revised 31 May 2011; accepted 11 June 2011)