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Interactive comment on "Oxygen isotope composition of final chamber of planktic foraminifera provides evidence for vertical migration and depth integrated growth" *by* Hilde Pracht et al.

Hilde Pracht et al.

brett.metcalfe@lsce.ipsl.fr

Received and published: 17 July 2018

Response to Reviewer 2. Takashi Toyofuku

Outlined below is our responses to their questions:

We thank the reviewer for their comments and thorough reviewing and will enact their textual suggestions. We do note however that at P14L17 Faber is not cited twice, it's a part 1 and part 2 paper.

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Discussion paper

Question 2 of P10L25, can you expect that the difference among size groups will become statistically significant as the number of samples increases?

Its an interesting question, the simplest answer is that we don't know whether by adding more data our results/significance would change. That being said, we do wonder if we expanded the size range to larger and smaller size classes whether we would see a different or a continuation of the trend. Or if we measured more specimens we would get a different result, essentially we can only know what we have measured with our current N and method. We know that, from a previous study, by increasing the number of size fractions or pooled analysis globally there seems to be a size isotope relationship (Ezard et al., 2015). Whether that holds true for individual sites or is the 'global' signal is intriguing.

Reference

Ezard, T.H.G., Edgar, K.M., and Hull, P., 2015. Environmental and biological controls on sizeâĂŘspecific δ13C and δ18O in recent planktonic foraminifera, Paleoceanography, doi: 10.1002/2014PA002735

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Discussion paper

Biogeosciences Discuss.,
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Biogeosciences Discussions

Interactive comment on "Oxygen isotope composition of final chamber of planktic foraminifera provides evidence for vertical migration and depth integrated growth" *by* Hilde Pracht et al.

Hilde Pracht et al.

brett.metcalfe@lsce.ipsl.fr

Received and published: 17 July 2018

Response to Reviewer 1. Jody Wycech

We thank the reviewer for their feedback and interesting comments, and apologize for not being able to have an online discussion regarding their points. Outlined below is our responses to their questions:

I recommend the authors also cite Kozdon et al. (2009). Kozdon et al. (2009) used SIMS to measure the d18O values of the ontogenetic calcite and reproductive crust of

N. pachyderma to reconstruct the species' water depth migration.

We shall include the paper at an appropriate spot.

Page 5 - Lines 20-22: How many G. ruber and N dutertrei were analyzed or were they just not weighed and measured for size? Perhaps breaking the description of T. Sacculifer analyses into a subsection within 2.1 will help (or changing the title of this section to be specific to T. sacculifer).

We shall add in an additional section as follows: "2.X Specific methodology for Question 3 To determine whether species of planktonic foraminifera from the same geographic location share the same or similar single specimen d18O(shell) variability specimens of G ruber (n = X) and N. dutertrei (n = X) were picked from the same interval. These shells underwent the same methodology outlined in section 2.1 for photographing, weighing and isotope analysis."

What were the additional steps used for T. sacculifers prior to stable isotope analysis? If the additional steps involve the heated block, the authors should also describe how the d18O analyses were performed for the G. ruber and N. dutertrei shells.

No all isotope values are based upon a heated block, the additional steps performed on T. sacculifer are outlined in sections 2.2 and 2.3. We shall add in the following text in line 22 (Pg. 5) so that it reads: "underwent additional steps, outlined in section 2.2 (dissection of chambers) and section 2.3 (size fraction), prior to stable isotope analysis."

Page 6 - Lines 20-21: What is the vital effect d18O correction? I think readers will be able to better interpret Fig. 6b with clarification. Is the correction necessary if you use the d18O-temperature calibration of Mulitza et al. (2003) instead of Kim and O'Neil (1997) (see comment regarding Page 6 – Line 30)?

We strictly use the equation by Kim and O'Neil as the definition for the d18Oeq, similar to previous studies by our and many other groups. The use of one equation to define (inorganic) equilibrium is preferred in order to avoid different equations for different

species or even for different shell sizes. Plankton pump and multinet collected specimens from the surface mixed layer were used to establish the value for the vital effect. For both species G. ruber and G. trilobus we used a d18Ove correction of -0.48 per mille. No vital effect correction was used for the non spinose species Neogloboquadrina dutertrei. These data are from Peeters et al., 2004 (Nature) and from Peeters et al., 2000 Ph.D. thesis). The error on the estimation of the vital effect is 0.15 per mille (1 s.d.).

Here we correct the measured values for vital effects of the species and perform statistical tests on both the corrected and uncorrected values. We will add in the following text: “In order to account for similar absolute measured values between species which are not produced by concurrent depth or seasonal preferences between species but instead by species-specific disequilibria from values obtained from ambient seawater equilibrium (so called ‘vital effects’) a correction was applied. The d18O shells were corrected by -0.48 per mil for G. ruber and G. sacculifer. “

Page 6 - Line 30: Why use Kim and O’Neil (1997) instead of a foram-based calibration such as Mulitza et al. (2003)? I suggest adding a sentence or two for explanation

See above: we want to avoid different equations and define strictly use K&O with an extra term for the vital effect. As such the temperature equations for different species may be offset from one another but all equations are parallel having a similar slope at a given temperature. We agree with the reviewer that a foraminiferal based calibration may be more suited in some instances, however these calibrations focus solely upon temperature as a driving factor for d18O (apart from the culture based ones). Carbonate ion is known to influence the d18O, and a number of these calibrations do not take this into account. Kim and O’Neil (1997) is used because it represents unmodified equilibrium, and whilst it also doesn’t take into account the CIE it is a useful base to build upon as it is not subjective. However, it is easy to vary the input using a number of published calibrations to test the sensitivity of these results.

Page 8 - Lines 7-9: I’m not sure how Figs. 5c-d show statistical significance (although the trend is quite obvious visually). Perhaps you could plot the 95% confidence interval on the robust regression (iteratively reweighted least squares) to show the slope is statistically different from zero. Alternatively, you could leave the plot as is and change the sentence to state D18O is dependent upon the d18O of the measured fragment.

We tested the significance (r being not zero) using Pearson’s correlation coefficient: The critical value for the absolute value of the correlation coefficient for alpha = 0.05 and N=57 (Deg. of freedom = 55) is 0.273 for DF=50 and 0.250 for DF = 60 (our table does not give a critical value for DF=55). Since our correlations coefficients are higher than these Critical values we may conclude they are different from zero. The correlation coefficients are also significant at the alpha = 0.01 level for which the highest critical values is 0.354 (for DF = 50). The graphs indicate that there is a relationship between the d18O value of the shell minus the final chamber and the d18O difference between the final chamber and the remaining shell value. This can be interpreted that the difference between final chamber and remaining shell may be a function of the surface water temperature as the (temperature difference between final chamber and shell minus final chamber) decreases with decreasing temperature. Potentially for low SST’s we may face a situation that the final chamber may even be warmer compared to the remaining shell value.

Page 9 – Lines 18-19: “above and below 100 m” is a bit vague. Note which water depths have temperatures of 24.5âŮęC and 25.5âŮęC at this site. If 25.5âŮęC measured from the <F fragment is not in the upper 0-50 m, that would suggest the fragment is also partly composed of GAM crust (see comment for Page 10 -Lines 18-21).

We chose to be a bit vague with the depths, however, we will add a section to the text considering the potential for the two proportions to have GAM addition. We will further consider that if GAM calcite precipitates upon the outside of the shell, then it could be that the proportion of GAM for F and <F may have different amounts if F has a larger surface area.

Page 10 – Lines 18-21: I've used image processing of shell walls in cross-section, which suggests that GAM crust composes 32-44% of T. sacculifer shells. I've also used SIMS to analyze the δ18O of PREGAM and GAM calcites in the penultimate chamber of Holocene and Pliocene T. sacculifer shells from several Atlantic and Pacific sites, and have found that the GAM is âĹij1‰ higher in δ18O than the PREGAM. That work is still in prep, but you could cite one of the following abstracts or my dissertation (refs below). I think Lines 18-21 undervalue the issues with GAM crust so at the very least, I suggest noting that the presence of GAM crust in the <F fraction may skew your results towards higher δ18O (colder temperatures and a deeper depth habitat).

In shells of living O. universa we have noted (after cracking shells and observing under SEM) that there is a linear relationship between the width of the inner wall and the width of outer wall (as delimited by the POM) in 'surface'-'mixed layer' samples that is not seen in the settling flux/dead population. We considered whether this reflects potential gam-calcite, therefore we look forward to seeing your results published and will include a citation.

This could explain why sometimes the last chamber is warmer compared to the remaining shell. However, if gam-calcite forms on the outer 'exposed' edges on the outer margin of the shell (and on the outer chambers) then the amount of GAM calcite would relate to the surface area exposed and how much GAM calcite may be added to the previous chambers (whole shell minus the last one) and the last chamber. Attached is a x-ray through a shell showing the complicated 'thickening'. We shall expand our discussion to incorporate this.

Page 11 – Lines 32-33: This section really highlights issues with the δ18O proxy, and suggests that the values do not reflect sea-surface conditions. I love the statistical methods used to reconstruct depth habitats, but the estimates seem too deep relative to culturing and plankton tow observations. For example, foram culturing scientists collect wild G. ruber and T. sacculifer from the upper 20 m of the water column so the probability of finding these species in the upper 50 m is not zero as your δ18O results

suggest. I recommend shifting the focus of the section away from where the forams are actually calcifying and focus instead on your δ18O results, why they deviate from field observations, and what this means for others who try to use δ 18O to infer ocean conditions.

Foraminiferal d18O does not reflect SST for two reasons, the first is that the vital effect leads to an offset and thus we must either alter the equilibrium line of KO or the individual d18O of the foraminifera. Secondly reflects the depth habitat of foraminifera: The depth habitat of foraminifera is a continuous variable however the calcification depths ('apparent calcification depths) represent a series of discrete intervals within the total depth habitat of a single specimen. Foraminifera construct a shell in which along the whorl the chamber size is 'exponentially' increasing in size, so that the cumulative fraction of each chamber to the total shell increases iteratively. Therefore, whilst foraminifera can be caught (via the authors own experience and as stated by the reviewer) in tows or via divers in the upper water column the seafloor shells are themselves skewed toward either a 'colder signal' or the signal with the greatest 'mass'. This mass balance approach can be seen, and is outlined, in Wilke et al., 2006. We completely agree with the reviewer that foraminifera do not catagorically record sea-surface conditions because of this 'weighted averaging'/cumulative mass balance.

I agree with the reviewer that "the probability of finding these species in the upper 50 m is not zero as your δ18O results suggest" and will alter both the text and figure accordingly, instead what these plots show is the depth of the apparent weighted average signal. One should interpret the zero probability above the interval with 'zero' probability as being part of the depth habitat, to make this more clear, we will mask the upper section to indicate that this should be seen as part of the depth habitat.

This brings us back to the reviewers comment regarding species-specific d18O equations, the culture derived d18O-temperature approximations took pains to remove the field-grown portion of the shell (via dissection and subsequent pooling of culture-grown chambers), with respect to other field based methods (such as from tows or pump

samples) this mass balance might skew the results.

ref: Wilke, I., Bickert, T., and Peeters, F.J.C., 2006. The influence of seawater carbonate ion concentration [CO32−] on the stable carbon isotope composition of the planktic foraminifera species Globorotalia inflata, Marine Micropaleontology,

Page 19 (Fig. 2): I suggest adding labels in the figure to identify the s.l. and s.s. morphotypes of G. ruber (or add a note into the figure caption which numbers are s.l. and s.s.).

Will be added in a revised version of the text

Page 21 (Fig. 4B): I think it may help to color code the points based on the morphologies noted in figure 2. I like the inset images and I think you should keep them, but I was curious about the morphology of the shells with high whole shell area and high final chamber area that didn't have a corresponding inset photo (points in the upper right)

We will endeavor to color code the points based on the morphologies noted in figure 2, but this might be a subjective approach (i.e. dependent upon the interpreter), we will see if another morphological parameter (such as deviation from a circle) could be used instead. However, we shall definitely add the morphology of the upper right.

Technical Corrections Even though the mass spec produces δ18O values to many decimal places, values beyond the tenth place are uncertain so d18O values should only be reported to the tenth decimal place (i.e., 0.1‰ precision).

Whilst, the reviewer is correct regarding isotope values there is also the problem of rounding error. We will add within the text a statement to that affect: "Isotope values are reported to 2 decimal places, however this should not be misconstrued as reflecting a degree of certainty but to prevent rounding error."

Page 1 - Lines 16-17: I suggest noting the direction of this difference, I.e. "We show that the d18O of the final chamber (δ18OF) is 0.2 ‰$\pm$0.4 ‰ (1σ) higher than the

d18O value of the test minus the final chamber (δ18O<F) of T. sacculifer"

We will modify the text accordingly

Page 1 - Line 17: Specify if sigma is standard deviation or standard error. Also, note how many shells you analyzed in the parentheses "(n=__)"

Sigma by itself is standard deviation, the symbol for standard error is sigma subscript SE or x-hat We will modify the text accordingly, by adding the n

Page 2 - Line 16: Remove the double parentheses

We will modify the text accordingly

Page 3 – Line 2: The word "do" is not necessary

We will modify the text accordingly

Page 4 - Line 10: Change "were" to "was" if only one test was performed (ANOVA with a post-hoc test)

A single test was performed, we will modify the text accordingly

Page 4 – Line 16: I stumbled over the statement "[...] our third objective Seasonality is a [...]"

We agree and have modified the text as follows:

"Having focused upon a single species for the first two objectives, our third objective focuses upon the variability of foraminifera isotope values, which are considered to represent seasonality, and whether fossil shells from different species have similar d18Oshell variability. "

Page 5 – Line 2: Condense the sentence to read "[...] same location, which would mean [...]"

We will modify the text accordingly

Page 8 - Line 16: I suggest separating these sentences to read something like "[. . .] Figure 6b). An ANOVA to test whether the species had equal means resulted in a p value of 0.0001 and led to a rejection [...]"

We will modify the text accordingly

Page 10 - Line 8: Change negative to positive (colder SSTs = more positive foram d18O values)

We will modify the text accordingly

Page 11 - Line 18: Add parentheses around the figure reference

We will modify the text accordingly

Page 10 - Line 28: Change the comma to a period

We will modify the text accordingly

Page 11 - Line 27-29: The verbiage is a bit awkward. I suggest dividing it into two sentences, I.e. "Wit et al. (2010) stated [...], which was inferred from measurements of single species (G. Ruber) at multiple core locations. Here we test [...]".

We agree, we will modify the text

Page 12 - Line 3: The verbiage is a bit awkward. I suggest, "First, we tested depth migration and found [..]". Page 12 - Line 7: Similar to line 3 I suggest, "Second, we tested covariance with size and found [..]". Page 12 – Line 9: If you use my suggestion for lines 3 and 7, this should be consistent, i.e. "Third, we tested [...]."

We will modify the text accordingly

Page 12 - Line 8-9: "found" is used twice in the sentence. I suggest deleting the second one, "[...] the three measured size classes."

We will modify the text accordingly

Page 12 – Line 10: Divide into two sentences. "[...] archives. Comparison between[...]"

We agree, we will modify the text

Page 12 – Line 21: Remove period after "BM"

We will modify the text accordingly

Page 17 – Line 31: Add superscript to 18 inδ18O

We will modify the text accordingly

Page 24 – Line 8: italicize the latin "in situ"

We will modify the text accordingly

Page 24 (Fig. 6 caption): Add in what the whiskers represent (e.g. 95% confidence interval) and if the horizontal lines within the boxes are the median or mean. The lines are typically medians, but the text compares means of the datasets so you may want to show both the mean and the median (perhaps one as a bold line and one as a dashed line?)

We will expand the caption to include a note regarding the various components, the central bar of the boxplots is the median, the top and bottom of the box the 25th and 75th percentile, the whiskers are 1.5*IQR +/- the 75th or 25th percentile. The 95 % CI on the median is not shown here, as that is a box and whisker with 'notch' plot.

Page 26, 27, 28 (Fig captions): Note what "p(δ18O)" is. I initially thought it was a p-value.

We will modify the text accordingly

Page 10 - Line 43: Use parentheses only around the year, i.e. "Berger et al. (1978b)"

Page 11 - Line 5: Use parentheses only around the year, i.e. "Brummer et al. (1987, 1986)"

Page 11 - Line 6: Use parentheses only around the year, i.e. “Peeters et al. (1999)”

Page 11 - Line 27-29: Use parentheses only around the year, i.e. “Wit et al. (2010)”.

Page 14 – Line 12: Remove the extra “, “

Page 17 – Line 24: Remove the extra “, “

We will correct these Endnote ‘cite as you write’ mistakes and modify the text accordingly

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Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-146, 2018.

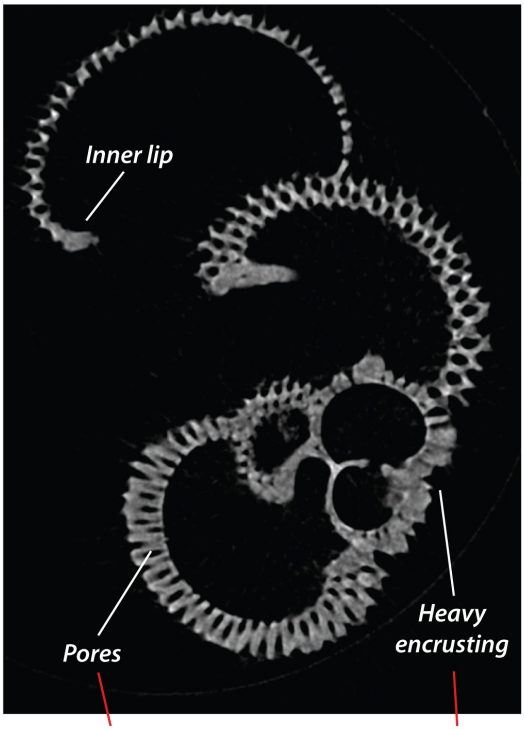

Fig. 1. Cross section of a shell of T. sacculifer

Oxygen isotope composition of final chamber of planktic foraminifera provides evidence for vertical migration and depth integrated growth

Hilde Pracht[1], Brett Metcalfe[1,2], Frank J.C. Peeters[1]

[1]Earth and Climate Cluster, Department of Earth Sciences, Faculty of Sciences, Vrije Universiteit Amsterdam, de Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands
[2]Laboratoire des Sciences du Climat et de l'Environnement, LSCE/IPSL, CEA-CNRS-UVSQ, Université Paris-Saclay, F-91191 Gif-sur-Yvette, France

Correspondence to: Brett Metcalfe (b.metcalfe@vu.nl)

Abstract. The translation of the original seawater signal (*i.e.*, ambient temperature and $\delta^{18}O_{sw}$) into distinct chambers of a single shell of a foraminifer during calcification can influence our interpretation of surface ocean conditions of the past, when based upon oxygen and carbon stable isotope geochemistry. In this study, we tested three different hypotheses related to: the size; the composition of the final chamber vs. the remaining shell; and species-specific offsets were tested to gain more insight into biological and ecological processes that influence the resultant composition of stable isotopes of oxygen ($\delta^{18}O$) in the shells of planktonic foraminifera. Shells of *Trilobatus sacculifer*, *Globigerinoides ruber* white and *Neogloboquadrina dutertrei* were picked from the top of multi-core GS07-150-24, of Modern age, offshore of North-East Brazil (3°46.474' S, 37°03.849' W) and analysed for single shell and chamber stable isotope analysis. We show that the mean value of $\delta^{18}O$ of the final chambers ($\delta^{18}O_F$) is 0.2 ‰ ± 0.4 ‰ (1σ) higher than the mean value $\delta^{18}O$ of the test minus the final chamber ($\delta^{18}O_{<F}$) of *T. sacculifer*. The formation of the final chamber approximately 1°C cooler than the chambers formed prior, suggests both ontogenetic depth migration to deeper water and a potential offset from the surface signal. Furthermore, we show that there is no statistical difference in the $\delta^{18}O_{sacculifer}$ values of shells of three different size classes, based upon measured size as opposed to sieve size, of *T. sacculifer*, although the pattern between the different size classes indicates depth migration during the life and growth of *T. sacculifer*. Comparison of vital effect corrected $\delta^{18}O_{shell}$ between *T. sacculifer*, *G. ruber* white and *N. dutertrei* suggests that *G. ruber* has a slightly shallower depth habitat (~ 90-120 m) compared to the other two species (~100-130 m). Disentangling depth versus seasonal habitat is complicated given the commonality between isotopes values from similar depths but different seasons, for instance the same average isotope value will have a shallower depth habitat in May than September. Calculation of seasonal-depth habitat was therefore tested. Our results highlight the complicated nature of interpreting oxygen isotopes even for the modern record.

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1. Introduction

1.1 Stable isotope values in Foraminifera

The oxygen isotope ratio in the shells of planktonic foraminifera ($\delta^{18}O_{shell}$) is used to reconstruct changes in water properties of the upper water column (*e.g.*, temperature, salinity, stratification) as well aid in palaeoclimatological reconstructions (*e.g.*, defining water mass characteristics, global ice volume, etc.). The knowledge of how this ratio is translated from the ambient environment into the shells of individual foraminifera is therefore important in order aid reconstructions and reduce associated error. The $\delta^{18}O_{shell}$ values recorded are a product of the temperature and the isotopic composition of seawater ($\delta^{18}O_{sw}$), itself a product of the evaporation and dilution (*e.g.*, precipitation, riverine runoff and ice melt) of seawater and hence directly correlated to salinity, which is further modulated by species specific preferences and metabolic effects (*i.e.*, vital effects). Reconstructions often utilize $\delta^{18}O$ produced from a number of pooled specimens, without reconciling how this impacts sample heterogeneity and therefore the resultant climatic interpretation (Figure 1). Assuming minimal disruption from sedimentary processes such as dissolution (McCorkle et al., 1997) or bioturbation (Hutson, 1980; Lougheed et al., 2017; Löwemark, 2007; Löwemark and Grootes, 2004; Löwemark et al., 2008; Trauth et al., 1997), the variance associated within a pooled $\delta^{18}O$ value is a product of the life histories of each individual that comprises the single measurement (Lougheed et al., 2017; Shackleton, 1967) and the underlying biological and ecological controls that govern such individuals depth distribution within the water column and seasonal occurrence (e.g. Peeters et al., 2002; Schiebel and Hemleben, 2017).

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1.2 Research Question and Hypotheses

In this manuscript we present the results of a number of experiments using single shells and dissected parts of single shells of planktonic foraminifera. Analysis of small quantities has been made possible with advances in techniques aimed at the routine measurement of microvolume amounts of CO_2 (Feldmeijer et al., 2015; Ganssen et al., 2011; Ishimura et al., 2012; Metcalfe et al., 2015; Scussolini et al., 2013; Takagi et al., 2015, 2016; van Sebille et al., 2015; Vetter et al., 2017; Wit et al., 2010; Wit et al., 2013). In order to evaluate the ecological and physiological impacts on the stable isotope values of foraminifera, three species of planktonic foraminifera (*T. sacculifer; G. ruber* white and *N. dutertrei*; Figure 2) were picked from a modern core top sample from the Tropical Atlantic Ocean (Figure 3). Given its gross morphology, in which individual chambers can be 'cleanly' dissected with minimal interference from other chambers (Lougheed et al., 2017; Shuxi and Shackleton, 1989; Spero and Lea, 1993; Takagi et al., 2015, 2016), several experiments were first performed on *T. sacculifer* (Figure 2i: 2vii). These experiments focused upon: (1) the differences between successive chambers (Lougheed et al., 2017; Shuxi and Shackleton, 1989; Spero and Lea, 1993; Takagi et al., 2015, 2016); (2) the size-isotope relationship of foraminifera, expanding upon Metcalfe, et al. (2015) and Feldmeijer et al. (2015) and (3) the difference in the variance between species. In the section below, we address three fundamental questions related to the oxygen isotope ecology of planktonic foraminifera. In the first question we aim to find out if there is evidence for depth integrated growth of calcite in a surface dwelling species. In the second experiment we focus on the question if shell size and oxygen isotope composition are correlated. Finally, for the third

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experiment, we investigate if the oxygen isotope composition of shells of different species from the same geographic location share the same variability.

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1.2.1 Question 1: Do individuals belonging to the species *T. sacculifer* calcify at one specific depth, or undergo depth migration?

The 'average' depth habitat of planktonic foraminifera of several species was first defined by (Emiliani, 1954) revealing that different species occupy discretely different depth habitats, independently corroborated by the later work of (Jones, 1967) by those same species presence and absence of species within the opening and closing nets. However, the offset in $\delta^{18}O$ measured between specimens growing within the euphotic surface waters and those collected from the seabed indicated that depth habitat is not confined to a single depth (Duplessy et al., 1981; Mix, 1987), instead this 'average' species depth habitat would be a weighted average of the various chamber calcification depths occurring during an individual's ontogeny (Kozdon et al., 2009a; Kozdon et al., 2009b; Shuxi and Shackleton, 1989; Takagi et al., 2015, 2016). Data from plankton tow studies combined with reproduction at depth would suggest that foraminifera migrate through the water column during ontogeny (Figure 1). For certain species of foraminifera (*i.e.*, *T. sacculifer* and *G. ruber*), however, a portion of the shell may have grown deeper in the water column than the living depths estimated by plankton tows (Lohmann, 1995), *i.e.*, either a calcite crust triggered by temperature change (Hemleben and Spindler, 1983; Hemleben et al., 1985; Srinivasan and Kennett, 1974) or reproduction triggered gametogenic calcification. For the first objective, we aim to test whether *T. sacculifer* performs depth migration, which would result in a deviation in the geochemistry between the different chambers of a single specimen and also in a deviation from the situation at the sea surface. A one-sample Student's t-test was used to test the claim that there is no difference between the mean of the final chamber and the remaining shell of *T. sacculifer*, *i.e.* the difference is equal to zero:

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Let $X = \delta^{18}O_F - \delta^{18}O_{<F}$

H_0: $\mu_X = 0$,

H_1: $\mu_X \neq 0$, (1)

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By computing the difference and using a reference value of 0, we do not invalidate the rule of independence that a two-sample Student's t-test would require between the two sample populations. This dependence is based upon the inference that μ_F and $\mu_{<F}$ could conceivably be considered to be 'before' and 'after' measurements and thus the value of $\mu_{<F}$ could have an impact upon the value of μ_F.

1.2.2 Question 2: Does the $\delta^{18}O_{shell}$ of *T. sacculifer* covary with size?

Our second research objective is an expansion of the first objective, as deriving palaeo-SST from the $\delta^{18}O$ compositions of foraminiferal shell is based on the assumption that a given specimen calcifies at, or produces a large proportion of its shell at, one specific depth in the water column. However, a portion of the variability associated with stable isotope measurements in foraminifera is believed to be size-dependent (Ezard et al., 2015). These size dependencies are typically attributed to biological effects and relate to depth migration through ontogeny (Feldmeijer et al., 2015; Metcalfe et al., 2015). For instance,

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investigations into the population dynamics of living specimens of *T. sacculifer* in the central Red Sea revealed that whilst this species in general occupies the upper 80 meters of the water column distinct size classes were shown to have clear depth preferences (Bijma and Hemleben, 1994; Hemleben and Bijma, 1994) with small foraminifera (100 to 300 µm) in the upper 20 m and progressive larger foraminifera with depth to the point that the largest specimens (>700 µm) lived between 60 m and 80 m. Calcification at different depths throughout their life span may cause a deviation in the $\delta^{18}O$ values of individuals from different sizes, depending on the ambient water column structure, which would therefore reflect different depths and thus the selection of an appropriate size fraction may or may not unduly influence palaeoclimate reconstructions. The aim of this second objective is to further expand upon the results of our first question and test whether the different depth preferences for different sizes of *T. sacculifer*, have an effect on the $\delta^{18}O$shell. Three size fractions were studied to learn more about the effect of size on $\delta^{18}O_{shell}$ and a one-way analysis of variance (One-way ANOVA) with a post-hoc test used to detect intra-sample differences was used to test the hypothesis that there is no difference between the means of the different size fractions of *T. sacculifer*:

H_0: $\mu_{small} = \mu_{medium} = \mu_{large}$,

H_1: at least one of the means is different, (2)

1.2.3 Question 3: Do different species of planktic foraminifera from the same geographic location share the same single specimen $\delta^{18}O_{shell}$ variability?

Having focused upon a single species for the first two research questions, our third question focuses upon the variability of foraminifera isotope values, which are considered to represent seasonality, and whether fossil shells from different species share similar $\delta^{18}O$shell variability. Commonly when referencing seasonality, temperature is considered as the variable of interest. However, the tropics have reduced temperature variation compared with higher latitudes, the core is situated along the northeast coast of Brazil which may be influenced by the shift in the ITCZ (Jaeschke et al., 2007). Temperature and salinity have opposing effects on the overall oxygen isotope composition. Surface dwelling species of planktonic foraminifera, *T. sacculifer* (Figure 2i: 2vii); *G. ruber* (Figure 2viii: 2xiii); and the thermocline dwelling *N. dutertrei* (Figure 2xiv: 2xix) were picked from the core top. All species are symbiotic (Schiebel and Hemleben, 2017) which limits the depth of the maximum growth. A One-Way ANOVA was used to test, whether the means of each species are equal or the if the alternative hypothesis that one or more of the species means differs from one another, with the following hypothesis:

H_o: $\overline{\mu_{T.sacculifer}} = \overline{\mu_{G.ruber}} = \overline{\mu_{N.dutertrei}}$,

H_1: $\overline{\mu_{T.sacculifer}} \neq \overline{\mu_{G.ruber}} \neq \overline{\mu_{N.dutertrei}}$, (3)

In addition to the ANOVA test for testing multiple means, we use a Kolmogorov-Smirnov (K-S) test to test whether the species stem from a similar distribution, with the claim in the null hypothesis being equal (i.e. not significantly different) distributions. Three tests were carried out: *T. sacculifer* vs *N. dutertrei*, *T. sacculifer* vs *G. ruber* & *N. dutertrei* vs *G. ruber*, respectively. For Objective 3, the hypothesis is that the $\delta^{18}O$shell variability varies for different species from the same location, which would mean that different species from the same location can give a different temperature and/or seasonality derivation (Mix, 1987;

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$H_0 : \sigma^2_{Species\ 1} = \sigma^2_{Species\ 2}$, ¶
$H_1 : \sigma^2_{Species\ 1} \neq \sigma^2_{Species\ 2}$, (4)¶
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Roche et al., 2017). Overall, the hypothesis is that different processes cause deviations from the sea surface equilibrium. More insight in the presence and size of this deviations can possibly be used to account for in future climate reconstructions.

2. Method and Material

2.1 Material and General Methodology

Multi-core GS07-150-24 was collected on board the Research Vessel G.O. Sars at a depth of 2412 m offshore the North-East of Brazil (3°46.474' S, 37°03.849' W; Figure 3). Following sub-sampling, the top of the core was washed over a >63 µm sieve, dried overnight, before being dry sieved over a 150 and 500 µm mesh. Regardless of the research question, each specimen underwent the methodological protocol of the VUA, this protocol aims to reduce uncertainty (*e.g.* specimen misidentification; anomalous or abnormal features) within single shell stable isotope analysis by cataloguing morphology and physical features of specimens prior to destructive analysis. After picking, the selected specimens were given a unique identifier, imaged in the umbilical position (Figure 2) using a Nikon Digital Research microscope with a Prior motorized stage. The motorized stage enables multiple images to be taken at pre-determined intervals in µm. These images were then combined using Nikon Digital Research D software into an Extended Depth of Focus (EDF) image. Each EDF image was then used to measure the diameter and surface area of both the final chamber and the whole shell, using the same programme. Groups of specimens were imaged together, with little impact upon the resolution (1 pixel, depending on the magnification, is equal to 0.3 to 1.5 µm) and placed into individual slides in order to generate a high throughput. After imaging, specimens were weighed individually in tin capsules using a Mettler-Toledo UMT microbalance (manufacturers precision 0.1 µg). In total 207 specimens of *T. sacculifer* were picked, weighed and measured for size. Following these measurements, specimens selected for research questions 1 ($\delta^{18}O$ difference between F and <F) and 2 ($\delta^{18}O$ difference between size) underwent additional steps, outlined in section 2.2 (dissection of chambers) and section 2.3 (size fractions), prior to stable isotope analysis.

For $\delta^{18}O$ and $\delta^{13}C$ analysis, shells and/or single chambers between 5 and 70 µg were placed in a 4.5 ml borosilicate exetainer vial, whereas shells between 20 and 145 µg were placed in larger 12 ml borosilicate exetainer vials (Breitenbach and Bernasconi, 2011; Feldmeijer et al., 2015; Metcalfe et al., 2015). Each vial was sealed with a cap with a pierce able septum, placed in a heated block (45°C), before being flushed with helium for 3 or 5 minutes to remove the ambient air (flow rate >100 ml/min.) depending on the size of the vial. Each sample was reacted with a few drops of phosphoric acid (H_3PO_4) for 160 minutes, transferred using a continuous flow of helium into a GasBench II preparation device, in which impurities were removed, before being introduced into a Thermo Delta$^+$ Mass spectrometer. Results were reported as δ values in per mil (‰), following voltage correction of the amplitude of mass 44 using grains of 150-180 µm of **V**rije Universiteit **I**nternal **C**arbonate **S**tandard (VICS: $\delta^{18}O$ = -5.44 ‰; $\delta^{13}C$ = 1.35 ‰) in order to be placed on the V-PDB scale. The precision of within-run international standards of IAEA-CO-1 and IAEA-CO-603 (minimum n = 10), placed to book-end every 6 samples, was better than 0.14 ‰ for both $\delta^{18}O$ and $\delta^{13}C$.

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2.2 Specific methodology for Question 1

To make inferences about depth migration (Research Question 1) 57 specimens of *T. sacculifer* were picked from two size fractions: 150 – 500 µm and >500 µm. Selection of specimens was based on the following criteria: (1) Specimens were intact, or did not appear externally to be broken or damaged; (2) Specimens were not visibly discoloured or overly contaminated with clay; (3) Specimens were not kummerform (Bé and Donk, 1971; Berger, 1969, 1970; Olsson, 1973), and/or it was possible for the sac-like chamber to be dissected; (4) Specimens and their final chambers were judged to be heavier than 6 µg to ensure sufficient mass for measuring on the mass spectrometer. Following the standard protocol, the final sac-like chamber was amputated (Shuxi and Shackleton, 1989; Spero et al., 1993; Ishimuru, 2012) from the rest of the shell with a number 7 dissecting scalpel, so that each shell was analysed in two portions, the last chamber ($\delta^{18}O_F$) and a shell without the last chamber ($\delta^{18}O_{<F}$). Those shells, minus the F-chamber, that still exceeded >150 µg were analysed in two parts. The remainder of the shell was placed between two glass slides, crushed, homogenised and then separated into two portions (identified as A and B). The isotope value of $\delta^{18}O_{<F}$ was calculated by using a weighted mean of the measured $\delta^{18}O$ from these two portions (*a* and *b*), with the following:

$$\delta^{18}O_{\mu<F} = \frac{(\delta^{18}O^{a}_{<F} \cdot amplitude^{a}) + (\delta^{18}O^{b}_{<F} \cdot amplitude^{b})}{(amplitude^{a} \cdot amplitude^{b})}, \quad (4)$$

Where, the amplitude is the amount of CO_2 of mass 44 produced in mVolts, which is linearly related to sample weight.

2.3 Specific methodology for Question 2

To make inferences about the effect of size on the measured isotopic composition (*Research Question 2*) 41 whole shells of *T. sacculifer* were picked from the >150 µm and >500 µm size fractions and subdivided based upon measured size. Three size classes were determined; Small: 222-316 µm (n = 10); Medium: 373-467 µm (n = 16) and Large: 511-597 µm (n = 15), the size classes have uneven widths with ranges of 94 µm; 94 µm and 86 µm respectively. The $\delta^{18}O_{shell}$ of *G. ruber* and *T. sacculifer* were corrected for their vital effect.

2.4 Specific methodology for Question 3

To determine whether species of planktonic foraminifera from the same geographic location share the same or similar single specimen $\delta^{18}O_{shell}$ variability specimens of *G. ruber* (n = 20) and *N. dutertrei* (n = 14) were picked from the same interval. These shells underwent the same methodology outlined in section 2.1 for photographing, weighing and isotope analysis.

2.5 Atlas data (Temperature, salinity and $\delta^{18}O_C$)

World Ocean Atlas 2013 (WOA13; (Boyer et al., 2013)) was used as an average climatology at the core site, temperature and salinity was extracted from the live access server (LAS) of NOAA. The oxygen isotope equilibrium values calculated by first computing the oxygen isotope of seawater ($\delta^{18}O_{sw}$) from WOA 13 salinity using the oxygen isotope database of LeGrande and

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Schmidt (2006). A regional mask was used on a global grid to define which regional equation to use, regions were redefined to fit established conventions on the definitions of particular ocean basins (similar to the approach of Roche et al. (2017)). Values of salinity that represent riverine outflow (PSU <10) were excluded from the resultant reanalysis of the salinity versus oxygen isotope of seawater relationship of the tropical Atlantic Ocean (LeGrande and Schmidt, 2006). Both WOA13 temperature and the computed $\delta^{18}O_{sw}$ were then used as input values for the equation of Kim and O'Neil (1997), rearranged from the relationship between temperature and the fractionation of oxygen isotopes in planktonic foraminifera, to derive the oxygen isotope equilibrium ($\delta^{18}O_{eq}$):

$$\delta^{18}O_{eq} = 25.778 - 3.333 \times (43.704 + T)^{0.5} + \delta^{18}O_{sw} \quad (5)$$

The use of Kim and O'Neil (1997) to define an, inorganic, equilibrium value of $\delta^{18}O$ is for a number of reasons, yet it is predominately to avoid different equations for (1) light-level; (2) foraminiferal size; (3) ontogenetic level (Bemis et al., 1998; Bemis et al., 2000); and (4) species (Mulitza et al., 1999b). In order to account for similar absolute measured values between species which are not produced by concurrent depth or seasonal preferences between species but instead by species-specific disequilibria from values obtained from ambient seawater equilibrium (so called 'vital effects') a correction was applied. The $\delta^{18}O$ values of *T. sacculifer* and *G. ruber* were corrected by 0.48 ‰ (1σ = 0.15 ‰; Peeters 2000; Peeters et al., 2004; and Peeters unpublished data). To understand the results a probabilistic determination of the seasonal-depth distribution using a fitted normal distribution to the single specimen data was calculated by fitting the probabilities of $\delta^{18}O_{shell}$ to the seasonal and depth distribution of $\delta^{18}O_{eq}$. Fitting was accomplished using a normal distribution, therefore to test whether the data comes from a normal distribution a K-S test (data normalised first) and Anderson-Darling test were performed. The probability determined for each $\delta^{18}O_{shell}$ is then transposed onto the $\delta^{18}O_{eq}$ of the core top.

3. Results

To aid the reader isotope values in the following section are reported to 2 or 3 decimal places, however this should not be misconstrued as reflecting a degree of certainty of the isotope values, but to report the results of the statistical without introducing rounding error.

3.1 Size vs. weight of *T. sacculifer*

During the picking and selection process, a total of 207 specimens of *T. sacculifer* was measured and weighted, generating data about size and weight. Ninety-eight of these were eventually analysed for stable isotopes, however data on size and weight for all 207 specimens was processed to make interferences about the relation between these two parameters (Figure 4). For comparison, the measured size and weight data was plotted alongside a theoretical hollow foraminifer (similar to *Orbulina universa*) in which the shell weight is calculated by assuming a constant porosity and the density of calcite is 2.71 kg^{-1} m^{-2}. This approach highlights the complexity when dealing with foraminiferal weight when both chamber number and chamber wall thickness is variable, there is a clear increase in the spread in shell weight (Figure 4a) when the area is greater than $4 \cdot 10^5$

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μm^2 this is likely either the result of chamber thickening or the growth process of foraminifera is not a linear process. After building the first chamber, a foraminifer builds a new chamber and while doing so, also adds a new layer of calcite to the previous chambers, making them a little thicker. This makes the weight increase deviate from a linear relation and also makes that the final chamber has less thick (and therefore lighter) walls than its predecessors (Bé and Lott, 1964). Regarding shell size vs. shell weight, a heteroscedastic relation was found. For smaller tests, little variance was present, deviation from the regression line increased when the area of the test increased, indicating more variability in shell weight for bigger shells. A possible explanation can be found in the fact that when shells grow bigger, they tend to get more divergent or wilder forms (Figure 4a), this especially goes for the final chambers. A relatively low weight in large specimens is then caused by a relatively large F. Because F has a relatively thin wall and therefore a low weight, the shell is lighter than expected (Bé and Lott, 1964). A relatively high weight in large specimens is caused by a big <F and small F. The chambers of <F have thicker walls and therefore a relatively high weight, causing a positive deviation from the size-weight regression line. A heteroscedastic relation also appeared between the area final chamber and the area of the whole shell. In Figure 4b it is visible that when the area of the whole shell increases, the variance becomes bigger; values become more scattered. Thus, as shells increase, there is a less clear relationship between total body size and the size of the final chamber. Compared to smaller shells, big shells tend to have relative small or big final chambers, which are not in proportion with the shell.

3.2 Question 1: Depth migration

Measured $\delta^{18}O$ values are plotted as a histogram in Figure 5a for $\delta^{18}O_{<F}$ and $\delta^{18}O_F$. The mean $\delta^{18}O$ values for the final chambers and the shells without final chambers, were ($\overline{\delta^{18}O_F} = \mu_F =$) -1.234 ‰ and ($\overline{\delta^{18}O_{<F}} = \mu_{<F} =$) -1.437 ‰ respectively, indicating the means of the two groups differ by approximately 0.203 ‰, with the final chamber having a more negative value than the shells without the final chamber. In Figure 5b, a histogram of $\Delta\delta^{18}O$, which represents the difference between $\delta^{18}O_{<F}$ and $\delta^{18}O_F$ is shown. The data is normally distributed, with a mean (difference) of +0.23 ‰ (Table 1). The one-sample t-test results in a *p* value of <0.05, therefore the null-hypothesis can be rejected at a significance level of a = 0.05, and we can conclude that the $\Delta\delta^{18}O$ is statistically different from 0. In other words, the difference between final chamber $\delta^{18}O$ and the $\delta^{18}O$ value of the shell with the last chamber removed, is positive and significantly different from zero at the 95% confidence level. The positive value indicates that growth of the final occurs, on average, at a lower lower temperature. A scatter plot between $\Delta\delta^{18}O$ and $\delta^{18}O_{<F}$ (r = 0.61; n = 57); and $\delta^{18}O_F$ (r = 0.69; n = 57) shows that there is a statistically significance between the variables (Figure 5c and 5d). The significance, of r being not zero, was statistically tested using Pearson's correlation coefficient. The critical value for the absolute value of the correlation coefficient for an α of 0.05 where n_1 = 50 is 0.273 and n_1 = 60 is 0.250. Our n_1 (= 57) taking into account the number of degrees of freedom (d.f. = n - 2) lies between these values of n_1. Since our correlation coefficients are higher than these critical values, it is possible to conclude that they are different from zero. The correlation coefficients are also significant for an α of 0.01 (C.V. = 0.354; for d.f. = 50).

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3.3 Question 2: Covariance with size

The mean $\delta^{18}O$ for the small, medium and large size fractions of *T. sacculifer* was -1.12 ‰, -1.30 ‰ and -1.15 ‰ respectively (Figure 6), with the smallest and largest shells having a less negative mean value than the medium shells. The resultant ANOVA-test *p* value of 0.136 (>0.05) however indicates that the null-hypothesis of equal means cannot be rejected, the observed differences between the different size classes are therefore not enough to state that there is a statistically significant difference between the mean $\delta^{18}O$shell of the small (222-316 µm); medium (373-467 µm) and large (511-597 µm) shells.

3.4 Question 3: Similarity in species specific variability for a single site?

The mean $\delta^{18}O$ of the single specimens of *N. dutertrei*, *T. sacculifer* and *G. ruber* were -0.84 ‰; -0.82 ‰ and -1.15 ‰ respectively (Figure 6b). An ANOVA to test whether the species had equal means resulted in a *p* value of 0.0001 which led to a rejection of the null hypothesis ($p < 0.05$) that the species have equal means. A post-hoc Tukey's all pairs comparison, using vital effect corrected $\delta^{18}O$ values, shows that the mean $\delta^{18}O$shell of *G. ruber* differed significantly from both *T. sacculifer* ($p = 0.0004$) and *N. dutertrei* ($p = 0.0017$) whereas the difference between *T. sacculifer* and *N. dutertrei* was not significant ($p = 0.9492$). Using the uncorrected, for vital effect, $\delta^{18}O$ values all species show statistical difference between one another. The range in species $\delta^{18}O$ is less than 1 ‰, from largest to smallest the range of *N. dutertrei* ($\delta^{18}O_{min.}$: -1.33 ‰; $\delta^{18}O_{max.}$: -0.46 ‰; $\delta^{18}O_{range}$: 0.86 ‰) is larger than *T. sacculifer* (min -1.20; max -0.39; range: 0.81%) and *G. ruber* (min. -1.55; max. -0.78; range 0.76) (Figure 6B). However, for three F-tests, to determine whether the species has equal variances, the resultant F value is less than the F-test Critical value and therefore the null-hypothesis that they have equal variances could not be convincingly rejected with the data measured. A Kolmogrov-Smirnov test was used to test whether the three-species come from the same distribution, which would indicate the three species have recorded the same climate signal. Three tests were carried out, each comparing the distributions of two species at the time. The test comparing *T. sacculifer* and *N. dutertrei*, resulted in a *p*-value of 0.9697 ($\alpha = 0.05$), meaning it is unable to reject the null-hypothesis. This means that we found no evidence that the two-species have a different probability distribution. For *N. dutertrei* vs. *G. ruber* ($p = 0.012$) and *T sacculifer* vs. *G. ruber* ($p = 0.030$) the null-hypothesis however, could be rejected therefore the two species record significantly different variability in $\delta^{18}O$.

4. Discussion

4.1 Depth migration

Numerous studies have subdivided the species *T. sacculifer* into the forms with a distinct final chamber, referred to as 'sac-like', from 'non-sac' forms referred to by its junior synonym *G. trilobus*. The division between these forms is not exclusively for studies with geochemical analysis but is also commonly found in studies using faunal abundance counts. In fact, a number of studies where *T. sacculifer* is used as a proxy for palaeoclimate have removed the final chamber to avoid potential bias

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caused by the assumed depth migration (Coadic et al., 2013). In our results we show a mean difference of approximately 0.203‰ between $\delta^{18}O_F$ and $\delta^{18}O_{<F}$, i.e. those forms that would be described as *T. sacculifer* and those as *G. trilobus*, with <F having more negative values (μ = -1.437 ‰) than F (μ = -1.234 ‰). A number of species of foraminifera, including the species analysed here (Bird et al., 2018), are associated with symbiotic algae that undergo diurnal migration into and out of the shell and vacuoles in the foraminifer's cytoplasm, a major function is their facilitation of both growth and longevity of an individual foraminifer (Anderson and Be, 1976; Be et al., 1982; Caron et al., 1981; Faber et al., 1988, 1989; Gastrich, 1987; Hemleben et al., 1989; Spero and DeNiro, 1987; Spero and Lea, 1993; Spero and Parker, 1985). As such the presence of symbionts places limits upon the range of depth habitat: juvenile foraminifer must either be re-infected by or capture new symbiotic algae (Hemleben et al., 1989; Spero, 1998) whilst adult foraminifera with symbiotic associations would do well to remain within the photic zone. Using the mean $\delta^{18}O_{sw}$ of the sample location of 0.42 ‰ (WOA13; (Boyer et al., 2013)) and the mean $\delta^{18}O$ of F and <F respectively, mean temperatures of 24.5°C and 25.5°C were derived which indicates a potential mean depth below and above 100 m respectively. The euphotic zone depth varies both regionally and temporally, from a lower limit of 20 m to greater than 120 m globally, with measured sites displaying variability between <40 m to >100 m on seasonal timescales (Buesseler and Boyd, 2009; Siegel et al., 2014). Spero (1998) reflecting on the evolutionary advantages for a species known to harbour symbiotic algae to calcify below the photic zone considered that there are none, and instead as planktonic foraminifera are at the mercy of ocean currents such specimens that reflect too deep growth (Lohmann, 1995) could represent descent or advection out of their suitable habitat range. In fact, our results highlight the complexity of the individual life histories of individual foraminifer like many species of (phyto- and or zoo-) plankton which are heavier than water (Huisman et al., 2002) their persistence within the upper water column, despite a sinking trajectory that should take them below conditions of light and nutrients sufficient for growth, may relate to turbulence and advection (Huisman et al., 2002; Margalef, 1978; Riley et al., 1949; Shigesada and Okubo, 1981; Sverdrup, 1953). Our results show that whilst the mean difference in $\delta^{18}O$, between F and <F, is weighted toward a colder signal within the final chamber there are however a number of shells that record a warmer signal in the F chamber (n = 8 for < -0.25 ‰; or n = 16 for < 0.0 ‰). Although the role of turbulence remains enigmatic (Davila and Hunt, 2001; Ruiz et al., 2004), with (Margalef, 1978) suggesting that favored species (*i.e.*, those with spines or bubble capsules) and size of specimens depends on whether turbulence is low or high, within a turbulent water column the overall population average may suggest a trajectory of a downward descent, whereas the descent of an individual shell maybe much more complicated. Our results suggest that there is a difference between chambers F and <F, on average the formation of the final chamber occurs in water approximately 1°C colder than the chambers formed prior, suggesting both ontogenetic depth migration to deeper waters and a potential offset from the surface signal.

The statistical significance between either the chambers F $\delta^{18}O$, and/or the <F $\delta^{18}O$, with the $\Delta\delta^{18}O$ (Figure 5c and 5d) could indicate that the environment in which the early chambers (<F) form determine the final chambers $\delta^{18}O$ (Figure 5c), the warmer SST (more negative $\delta^{18}O$ values) specimens have a larger $\Delta\delta^{18}O$ which could indicate the specimen lived during stratified water conditions (Figure 1b). Likewise, the colder SST (more positive $\delta^{18}O$ values) specimens have a smaller difference, therefore these specimens could represent those that live under mixed conditions (Figure 1a). Specimens that show a warming

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between F and <F chambers could theoretically have calcified during a period of change, a transition from a stratified to a mixed (or vice versa) water column.

4.2 Difference between F and <F: an underestimation?

The difference in the isotopic value between successive chambers may not depend solely on depth migration during ontogeny but may be altered by chamber thickening. Two types of chamber thickening are known to exist: a calcite crust seen in Neogloboquanids and Globorotalids; and gametogenetic calcite (GAM) seen in *Orbulina universa* and *T. sacculifer*. Whilst both types are produced at the end of the life cycle and therefore deeper in the water column, one is considered to represent low temperature thickening of the shell whereas the other a pre-reproduction thickening of the shell. Thickening of the pre-existing chambers that compose a single shell in response to a particular environmental parameter or at the end of the life cycle may bias the resultant isotopic composition, depending on the water column structure and depth of the mixed layer (Figure 1 A and B) the calcite produced in such a way may be indistinguishable isotopically from older chambers.

. Whilst, the size of this bias induced by GAM may have been overestimated in the literature, for instance using cultures (Hamilton et al., 2008) showed that approximately 80% of the shell material is pre-GAM., new evidence suggests that the $\Delta\delta^{18}O$ between Pre-GAM and GAM is ~1 ‰. (Wycech et al., 2013; 2016; Wycech, 2017) The same work suggests that GAM comprises 32 to 44% of *T. sacculifer* shells. Determining how much of the <F and F chambers are altered by GAM is complicated because GAM calcite precipitates on the outer 'exposed' edges/margin of the shell, the amount of GAM relates to the surface area. Now, by removing the final chamber, a section of this surface area would not have been exposed during GAM formation. Therefore, the size of the over-printing is a product of both the amount of GAM calcite and the surface area exposed. Our results should therefore be considered as the minimum deviation between <F and F.

4.3 Covariance with size

The trends in size-isotope values have been grouped into what (Berger et al., 1978b) considered to be three types: "normal" showing enrichment with increased size; "reversed" showing depletion with increased size and "mixed" in which neither enrichment or depletion with increasing size occurs. From our data there is no statistical difference in the $\delta^{18}O$ of the three different size classes, despite the appearance of a 'mixed' signal, meaning there is absence of evidence to state that the $\delta^{18}O$ of *T. sacculifer* is subjected to a size effect. Evidence from our final chamber comparison shows that individuals undergo depth migration. Berger (1978) considered that such a scenario should result in a 'normal' size-isotope trend, however depth migration with size is not demonstrated in the $\delta^{18}O$ of the distinct size fractions. Berger (1978) further considered that the 'mixed' trend poses a problem in the interpretation of $\delta^{18}O$ solely in terms of depth migration. However, a study of population dynamics in the Red Sea indicate following reproduction at depth from the preceding generation, juveniles ascend in the water column to mature, where after these maturing foraminifera descend when reaching the reproductive size (Bijma and Hemleben, 1994; Hemleben and Bijma, 1994). How small forms would migrate from depth is unknown, although ascending particles due

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to low density do exist in the marine environment (Azetsu-Scott and Passow, 2004; Mari, 2008; Mari et al., 2007). This would lead the smallest shells to have calcite that was formed in deeper, colder waters, medium sized shells to consist of calcite formed at the surface, in warmer waters and larger shells formed of calcite from deeper, colder waters. One caveat to such a scenario is that (Brummer et al., 1987, 1986) considered juvenile-neanic stages of the planktonic foraminiferal life cycle to be less than 100-200 µm, distinctly smaller than the shell sizes measured here. Peeters et al. (1999) have shown that the size frequency distribution associated with the adult population of numerous planktonic foraminiferal species is distinctly gaussian in shape and thus variance around the mean should be considered as 'dwarfs' and 'giants' (Berger, 1971), thus a mixed signal may reflect extra-seasonal growth. A point of caution with size-isotope trends is that (Metcalfe et al., 2015) previously showed that such trends can either be consistent down core (e.g. *G. truncatulinoides*) or varying (e.g. *G. bulloides* and *G. inflata*) and therefore upscaling one relationship either spatially and/or temporally may lead to erroneous results.

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4.4 Species specific variability

The comparison between the δ^{18}Oshell values of the three species, demonstrated that *G. ruber* (µ = -1.15 ‰) has a different mean δ^{18}O value and a different δ^{18}O distribution than either *T. sacculifer* (µ = -0.82 ‰) or *N. dutertrei* (µ = -0.84 ‰). Solving the palaeotemperature equations for each species using the mean δ^{18}O values gives equivalent temperature of 24.1 °C for *G. ruber*, 22.6 °C for *N. dutertrei* and 22.5 °C for *T. sacculifer*. This suggest a difference in depth habitat and/or season of growth between *G. ruber* and the other two species furthered highlighted by a comparison with the annual average and cumulative distribution functions (CDF) for specific depths (Figures 7B and 7C). Disentangling the signals of depth migration from seasonal habitat is complicated given the commonality between isotope values from similar depths and different seasons and vice versa. For instance the same average isotope value will have a shallower depth habitat in May than in September. To illustrate this, at two specific depths (100 m and 125 m; based on Fig. 6C), the $\delta^{18}O_{shell}$ of the foraminifera, corrected for the vital effect, was compared to the $\delta^{18}O_{eq}$ over the year for a number of discrete depth levels in the water column, to find out at which depth level(s) a given species could grow, assuming a uniform shell flux over the year (Figure 7A). For *N. dutertrei* and *T. sacculifer* these potential depths and seasons of growth are similar, following from the fact that their mean $\delta^{18}O_{shell}$ and $\delta^{18}O_{shell}$ variability is not significantly different. It was found that *T. sacculifer* and *N. dutertrei* could potentially occur year-round at ~125 m and at ~100 m from respectively August to December and February to June. *G. ruber* in its place, reflects the year-round temperature at 100 meters of depths, and autumn/winter temperatures (August to December) at a depth of 125 meters. Wit et al. (2010) stated that the variability within single shell δ^{18}O measurements could be a proxy for seasonality (Ganssen et al., 2011; Vetter et al., 2017) which was inferred from measurements of single species (*G. ruber*) for multiple core locations to test this inference. Here we tested whether different species are influenced by seasonality in a similar or dissimilar way.

Our results imply that species can be used as indicators for year-round seasonality, because the variability in single shell δ^{18}O matches the variability in annual temperature derived from the climatological average of WOA13, but only at species-specific depths (*T. sacculifer* and *N. dutertrei* for 125 m, and *G. ruber* for 100 m). The probability plots of the season-depth habitat, as

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indicated by Figures 8-10, show that the calcification depth recorded by the shell $\delta^{18}O$ is a narrow interval between 50 and 200 m. Despite evidence for the contrary, $\delta^{18}O$ does not implicitly record sea surface temperatures, collection of foraminifera by SCUBA (Bird et al., 2018; Spero, 1998) and net collection (Ottens et al., 1992; Kroon and Ganssen, 1989; Ganssen and Kroon, 1991) at or in close proximity to the sea surface represents a part of, but not their full, life cycle. This situation is further exacerbated by both a shallow or deep mixed layer giving a potential homogeneous $\delta^{18}O$ signal from surface to deep (Figure 1) and the unknown quantity of the vital effect when attempting to derive depths from core top material. It is worth reiterating, here, several conclusions of previous studies (Wilke et al. 2006). Foraminiferal depth habitat is a continuous variable from zygote fusion to eventual reproduction induced mortality. However, chambers represent a distinct event covering a short period of time (~12 hrs), the calcification depths of chambers therefore reflect discrete intervals along this continuous depth habitat. As chamber size increases progressively, in normal forms (Berger 1969), from the earliest to the final chamber the contribution of each chamber to the cumulative signal increases iteratively and can be approximated by a mass balance (*e.g.* Wilke et al., 2006). As the shell sinks through the water column, during its life, the signal will become progressively skewed toward a deeper 'colder' signal. Modification of this signal via crust formation or GAM-calcite will bias the signal further toward higher $\delta^{18}O$ and a colder signal. The depth habitat of foraminifera is not static globally, instead its dynamism represents a complex interaction between food, temperature, water column structure and where appropriate light. Discrepancies between previously published work should not be considered in depth but on the various attributes of the water column present, as it those parameters altering with depth that ultimately allow foraminiferal growth to occur.

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5. Conclusions

To gain more insight into biological and ecological processes that influence the $\delta^{18}O_{shell}$ of planktonic foraminifera, three research questions with associated hypotheses were tested. First, we tested depth migration and found that a significant difference in $\delta^{18}O$ between the final chamber ($\delta^{18}O_F$, μ = -1.23 ‰) and the test minus the final chamber is observed in *T. sacculifer*. This difference in $\delta^{18}O$shell is equal to a temperature difference of 1 °C, suggestive that the final chamber is formed via depth migration in waters that are approximately 1 °C cooler than the chambers formed prior. Second, we tested covariance with size and found that despite evidence for depth migration during the life and growth of *T. sacculifer* there is an absence for a size effect on *T. sacculifer* with no statistical difference in the $\delta^{18}O$shell of the three different size classes. Third, we tested species-specific $\delta^{18}O$ variability to quantify the effect upon the populations from proxy archives. Comparison between *T. sacculifer* (μ = -0.82‰), *G. ruber* $_{white}$ (μ = -1.15 ‰) and *N. dutertrei* (μ = -0.84 ‰) indicate that *G. ruber* has both a significantly different mean and variability in $\delta^{18}O$, suggestive that the species lives in warmer shallower waters (i.e. ~ 90 – 120 m vs. ~100 – 130 m). However, inferences about depth and/or seasonal habitat is complicated by the fact that similar $\delta^{18}O_{eq}$ values occur in both time and depth. It is possible, based upon our results that *T. sacculifer* and *N. dutertrei* could potentially occur year-round at 125 meters of depth and at 100 meters of depth from respectively January to August and February to June. *G. ruber* in its place, reflects the year-round temperature at 100 meters of depths, and autumn/winter

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temperatures (August to December) at a depth of 125 meters. These results highlight the complicated nature of interpreting oxygen isotopes even for the modern record in line with previous findings (Kretschmer et al., 2018; Roche et al., 2017). Depth migration, size and species -specific variability all influence the values of $\delta^{18}O$ within a foraminiferal shell and therefore the resultant palaeoclimate reconstructions conclusions drawn from their isotope values.

Author Contributions

FJCP and BM designed the study and HP performed data collection and analysis under supervision of BM and FJCP, upon material collected by FJCP. HP and FJCP performed statistical analysis. BM produced the figures. All authors contributed to the writing of the manuscript.

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Author Contributions

The authors declare they have no conflicts of interest.

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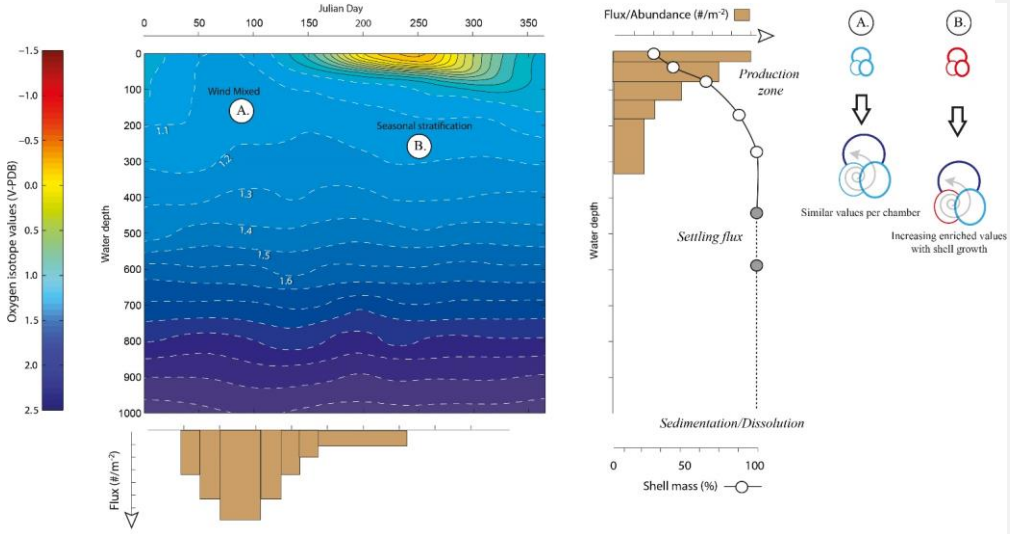

Figure 1: Schematic to show the competing individual and population dynamics that may contribute to the variance within the $\delta^{18}O$ of a population. Growing seasons maybe exaggerated or minimized by water column properties (*i.e.*, rapid population turnover reflecting 'bloom' conditions) as highlighted by the uneven widths of the flux histogram. Likewise, rapid growth during juvenile chamber formation may lead to larger offsets (Berger et al., 1978a; Mulitza et al., 1999a) as well as large differences between the surface and deep equilibrium $\delta^{18}O$, however this is offset by the percentage that these juvenile chambers contribute to the whole shell $\delta^{18}O$. This is particularly influential if scenario B (a stratified water column) occurs rather than scenario A (a well-mixed water column). Schematic modified from Metcalfe et al. (2015).

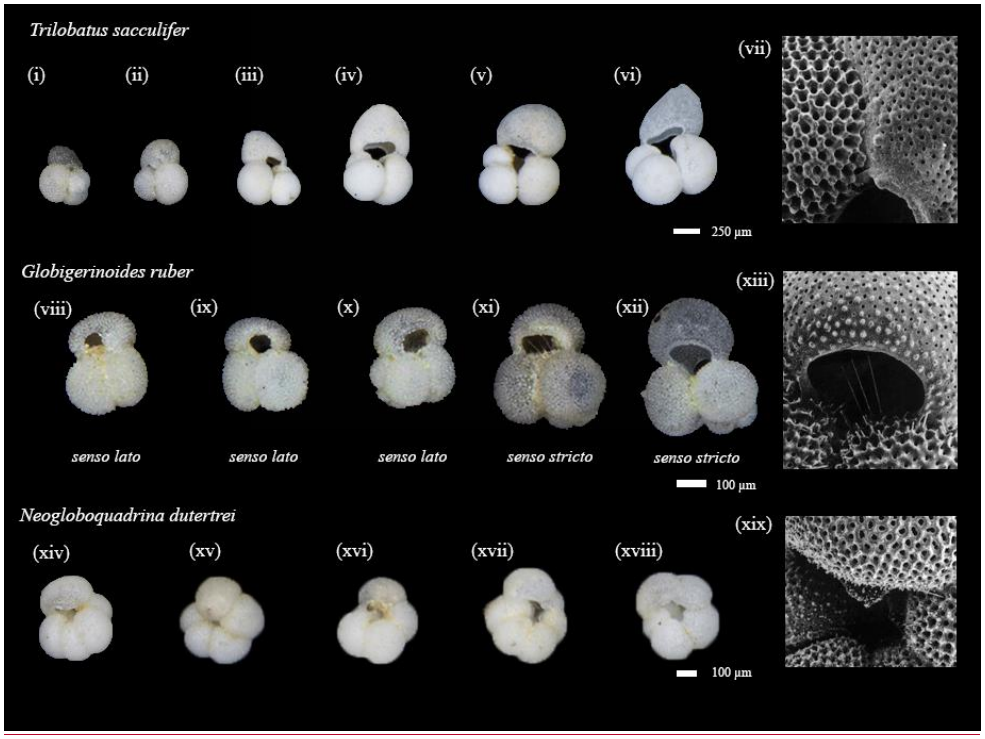

Figure 2: Representatives of species used within this study. Light microscope from core top location and scanning electron microscope (SEM) images used to highlight particular features were collected using plankton tows and plankton pumps from the Arabian Sea during the NIOP cruise (Peeters, 2000; Peeters et al., 2002). Note that this final sac-like chamber of *Trilobatus sacculifer* has various unique morphologies including a thinner walled variety giving the specimen's F chamber a translucent quality (similar to i. and vi.). The species *Globigerinoides ruber* has two morphotypes referred to as (xi – xii) senso stricto (s.s.) and (viii – x) senso lato (s.l.). Whilst *Neogloboquadrina dutertrei* is distinguished from other species of *Neogloboquanids* by the presence of a 'tooth'.

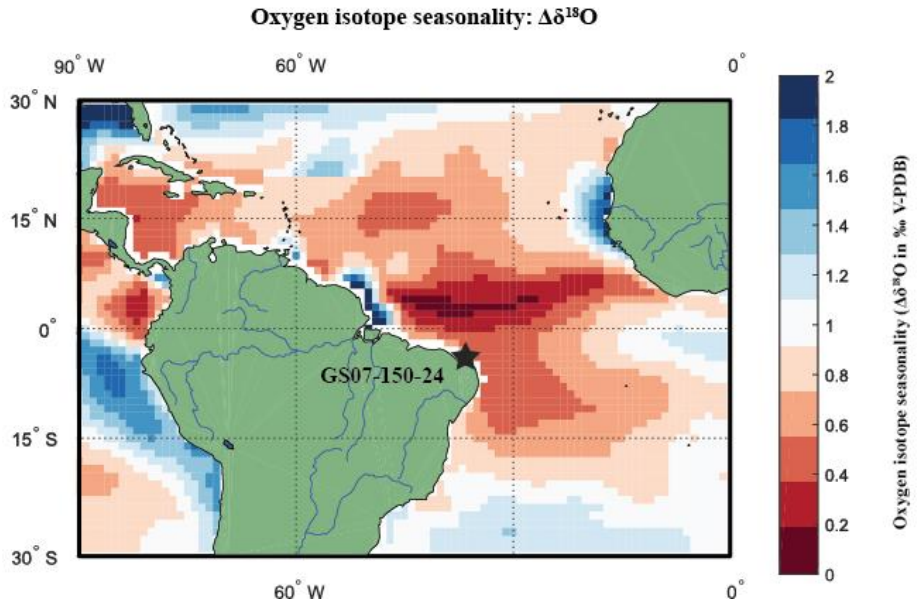

Figure 3: Location Map of RETRO multi-core GS07-150-24 plotted on a basemap of sea surface $\delta^{18}O_{eq}$ seasonality. Location of multi-core (black diamond) plotted on a seasonal oxygen isotope equilibrium ($\Delta\delta^{18}O$) base-map, calculated by subtracting the maximum and the minimum ($\delta^{18}O_{eq}$) of WOA13 temperature and salinity data converted into input variables for a rearranged (Kim and O'Neil, 1997) equation. Core location has an estimated $\Delta\delta^{18}O$ of 0.6 ‰. Note that the coast line basemap of WOA13 is of a far lower resolution than Mathworks MatLab® 2016 Mapping toolbox, thus white areas around the coast represent a lack of data.

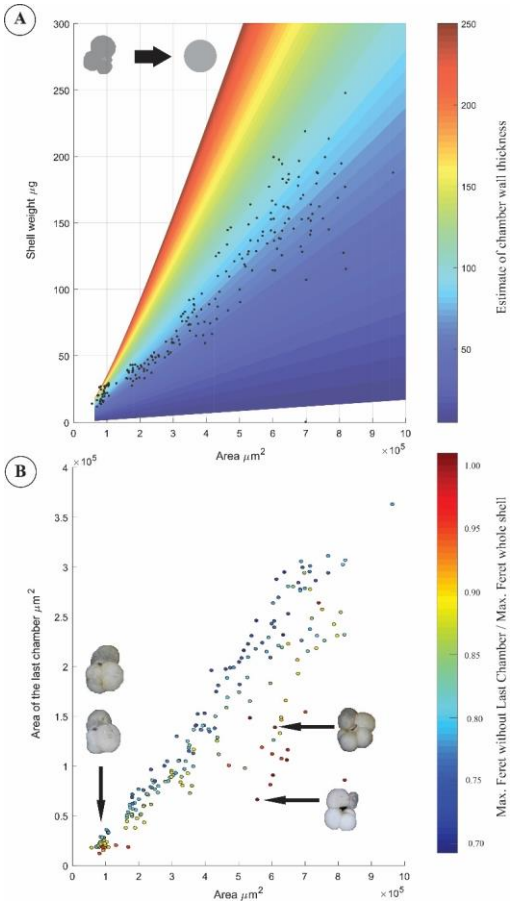

Figure 4: Physical properties of *T. sacculifer*. (A) Size vs. Weight of *T. sacculifer*, the data is overlaid on a theoretical calculation of what would the shell weight be of a spherical hollow foraminifer with consistent porosity, assuming the density of calcite. Colour represents variation in wall thickness used to calculate the difference between the inner and outer sphere volume. (B) The area of the final chamber vs. the whole shell area of specimens measured, pictures inset highlight the morphology associated with the spread

in the datasets. The scatter points filled colour reflects the ratio between the maximum ferret diameter with and without the last chamber. The linear regression equation is y = 0.0002x -4.5243 (r^2 = 0.8985; n = 207)

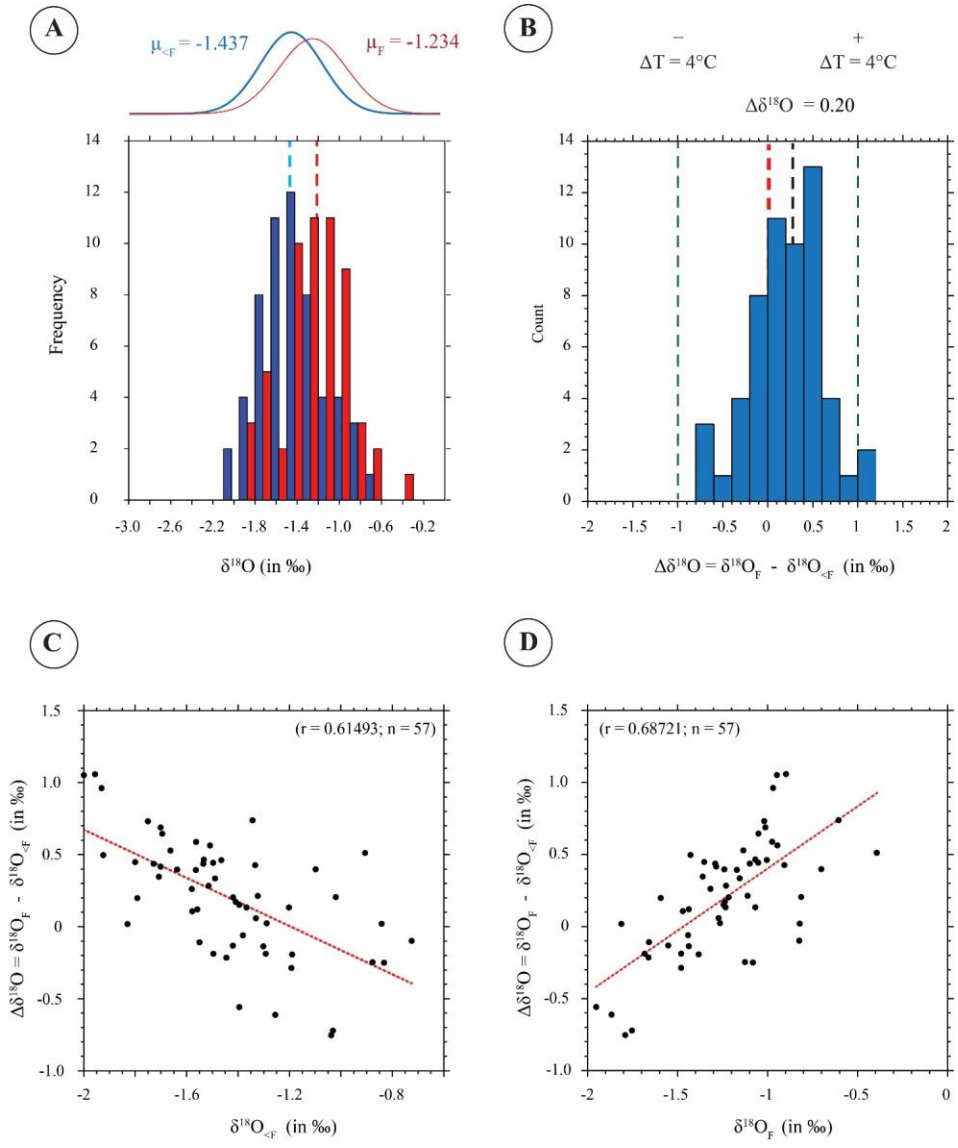
A
μ<F = -1.437
μF = -1.234
Frequency
δ18O (in ‰)
B
−
+
ΔT = 4°C
ΔT = 4°C
Δδ18O = 0.20
Count
Δδ18O = δ18OF - δ18O<F (in ‰)
C
(r = 0.61493; n = 57)
Δδ18O = δ18OF - δ18O<F (in ‰)
δ18O<F (in ‰)
D
(r = 0.68721; n = 57)
Δδ18O = δ18OF - δ18O<F (in ‰)
δ18OF (in ‰)

Figure 5: Final chamber vs. rest of shell $\delta^{18}O$. (A) Raw $\delta^{18}O$ values of rest of shell (blue) and final chamber (red) plotted as a histogram, vertical bars represent sample means. Fitted normal distributions for rest of shell (μ<F; blue) and final chamber (μF red), with mean average values are also indicated. Histogram bins are in 0.25 ‰ bin intervals, the equivalent of ~1°C depending on whether the data is on the high or low end of the scale. (B) Histogram of the same specimen difference in $\delta^{18}O$ (≡ $\Delta\delta^{18}O$) between <F and F. Vertical green lines at $\Delta\delta^{18}O$ of ±1 ‰ represent a ~4°C temperature variation, a vertical red line denotes no difference ($\Delta\delta^{18}O$ = 0). The average $\Delta\delta^{18}O$ (μ = 0.203) is shown as a black vertical line. (C & D) Scatter plot of the same data plotted as either the (C) <F remaining of the shell and (D) the F chamber $\delta^{18}O$ vs. $\Delta\delta^{18}O$. All values in per mil (‰) on the V-PDB scale.

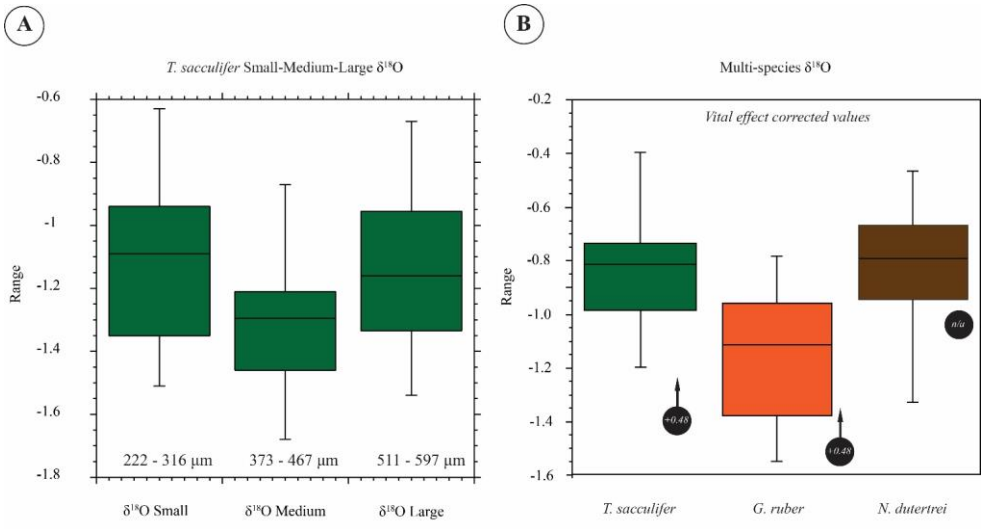

Figure 6: Box-plots of the oxygen isotope values ($\delta^{18}O$) versus size and for different species of planktonic foraminifera. (A) Small, medium and large (see text for definition of sizes represented, note uneven size interval). (B) Pastel colours (right hand side) box-plots are corrected for vital effects (circles with arrows) calculated from *in situ* water sampling (plankton pump and plankton tow; Peeters *unpublished*). The central bar of each box represents the Median, The top and bottom of the box are the 25th and 75th percentile of the data, whilst the whiskers are calculated from (1.5*IQR) ± 25th or 75th percentile.

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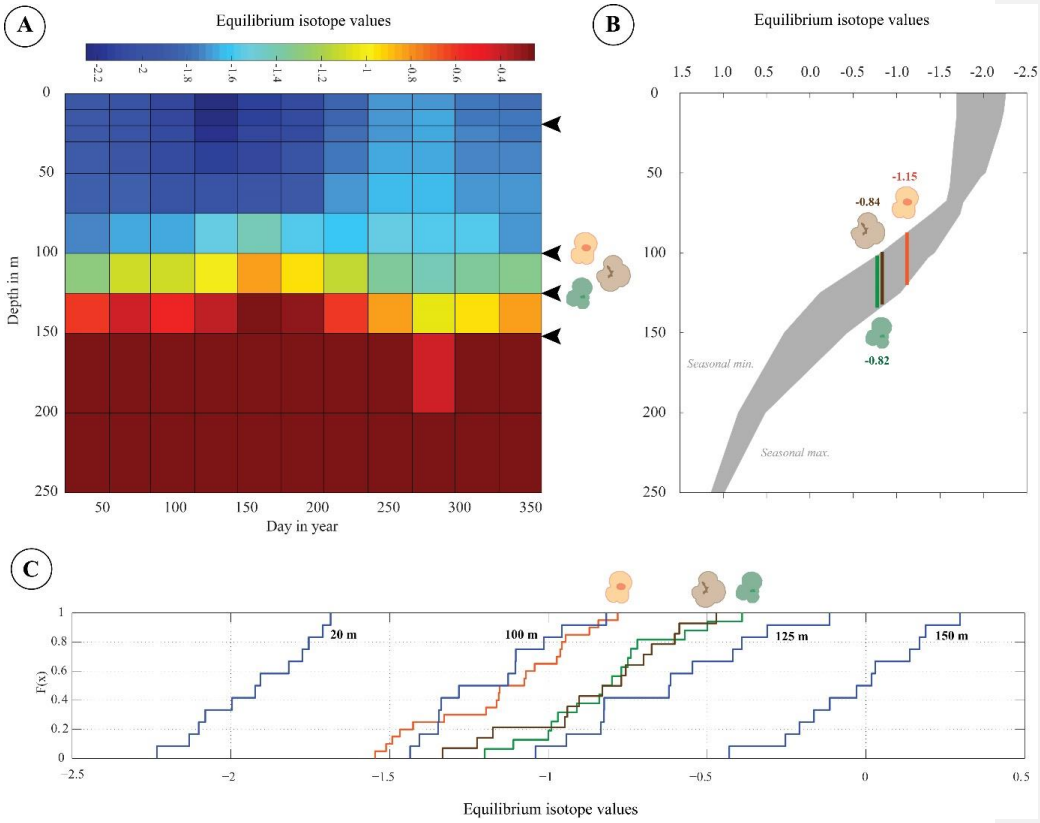

Figure 7: Season or depth, predicting likely depth habitats of planktonic foraminiferal species using inferred equilibrium oxygen isotope values ($\delta^{18}O_{eq}$). World ocean atlas (WOA13) temperature and salinity was used to compute the equilibrium oxygen isotope value using the Tropical Atlantic $\delta^{18}O$-salinity relationship defined by (LeGrande and Schmidt, 2006) input into a rearranged form of (Kim and O'Neil, 1997) (See section 2.4). (A) Contour plot of $\delta^{18}O_{eq}$, plotted as depth in m versus day in year. Note the uneven depth interval distribution inherent within the WOA dataset. Black arrows represent depths chosen in (C) to calculate cumulative distribution, species symbol represent inferred depths of mean average values. (B) The seasonal minimum and maximum (grey band) in $\delta^{18}O_{eq}$ for each depth interval, coloured bars represent depth intervals that are similar to isotopic values of the three species. (C) Cumulative density distribution of the three-species plotted alongside the CDF for 20, 100, 125 and 150 m distributions, values are plotted as probability (F(x)) versus Oxygen isotope value. All values plotted in per mil (‰) on the V-PDB scale.

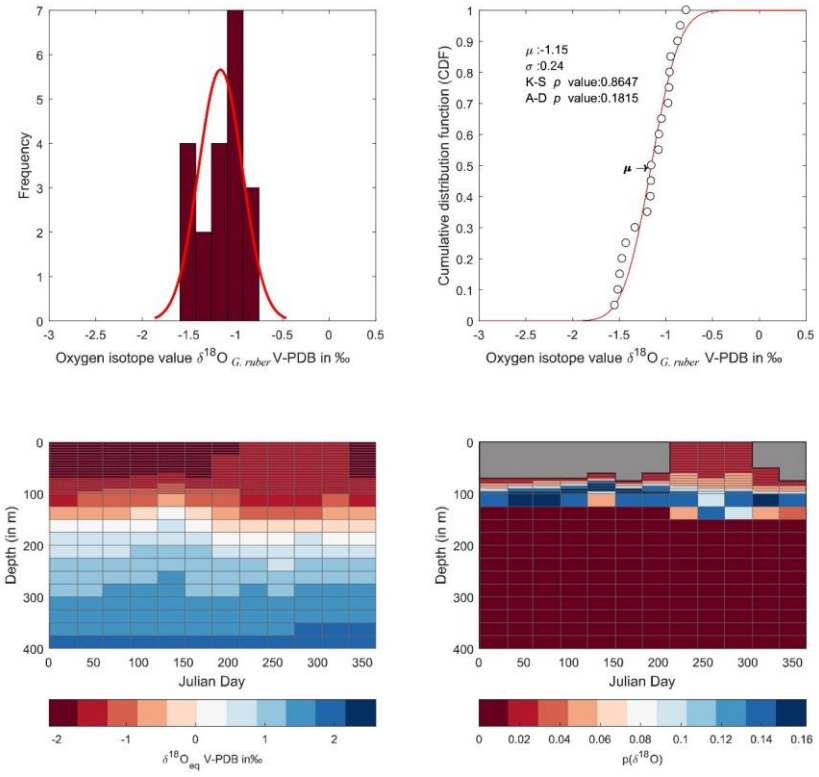

Figure 8. Calculated $\delta^{18}O$ probability ($p(\delta^{18}O)$). (*Top left*) Single specimen isotope measurements for *G. ruber*, with a fitted normal distribution. (*Top right*) This data is used to produce a cumulative distribution function plot (CDF), with statistical output for an Anderson-Darling test, and a Kolmogorov-Smirnov test following data normalisation (failure to reject the null hypothesis at the 5% confidence suggests the data is not statistically different from a standard normal distribution, red line in plot). (*Bottom left*) In situ $\delta^{18}O_{eq}$ values predicted from a regional-specific equation (LeGrande and Schmidt, 2006) and a rearranged form of (Kim and O'Neil, 1997) with WOA13 temperature and salinity values as input values. (*Bottom right*) Resultant calculated $p(\delta^{18}O)$, the probability of each discrete $\delta^{18}O$ is denoted as $p(\delta^{18}O)$ mapped upon the $\delta^{18}O_{eq}$ WOA13 values. The grey region represents the area between 0 m and the first probable depth that may have become overprinted during depth migration.

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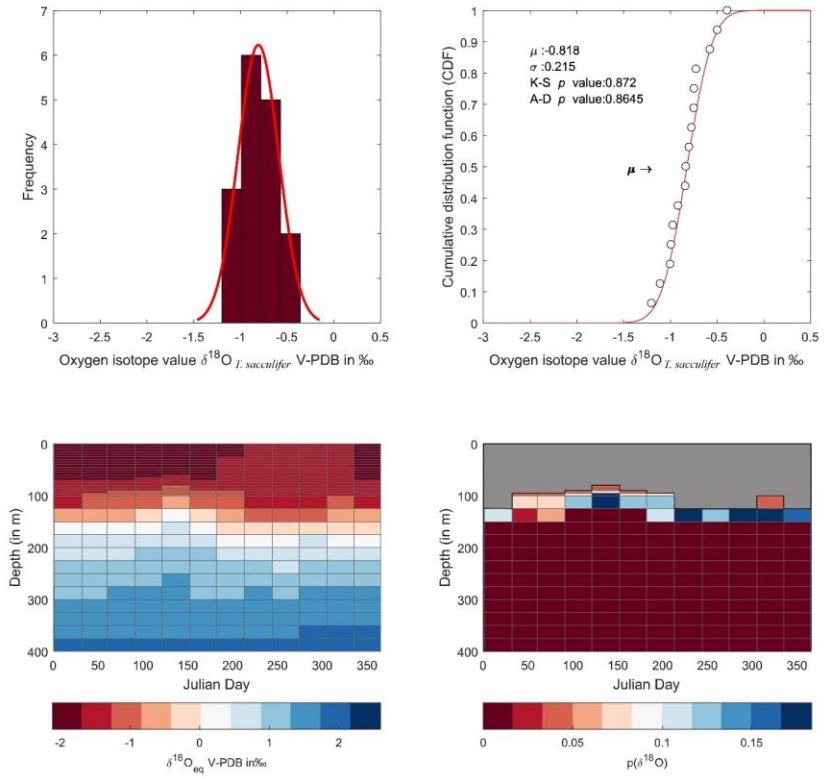

Figure 9. Calculated $\delta^{18}O$ probability ($p(\delta^{18}O)$). As per figure 8, with individual isotope values, CDF distribution, WOA13 $\delta^{18}O_{eq}$ and resultant $p(\delta^{18}O)$ but with *T. sacculifer*.

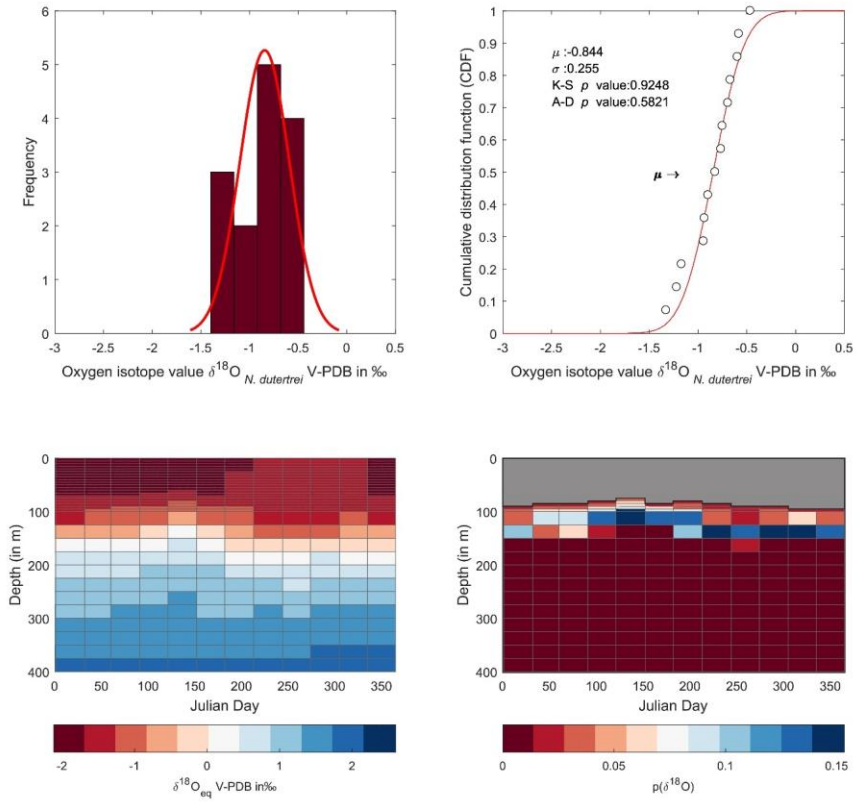

Figure 10. Calculated $\delta^{18}O$ probability ($p(\delta^{18}O)$). As per figure 8, with individual isotope values, CDF distribution, WOA13 $\delta^{18}O_{eq}$ and resultant $p(\delta^{18}O)$ but with *N. dutertrei*.

Count	**57**
Mean	0.20
Variance	0.16
Std. Dev	0.40
Std. Err	0.054
Mean difference	0.20
Degrees of Freedom	56
t Value	3.78
t Probability	0.0004

Table 1: Results of the Student t-Test for a single group. Testing the $\delta^{18}O_f$ -$\delta^{18}O_{shell\text{-}f}$. Test value: 0 (testing whether the difference between $\delta^{18}O_f$ and $\delta^{18}O_{shell\text{-}f}$ is statistically equal to 0).

Analysis of Variance Results					
Source	**Degrees of freedom (DF)**	**Sum of squares (SS)**	**MS**	**F value**	**P value**
Total	40	2.49	0.06	2.12	0.13
A	2	0.25	0.13		
Error	38	2.24	0.06		

Tukey's All Pairs Comparison				
	Mean difference	|q|	P	95% CL
$\delta^{18}O_{shell}$ small vs $\delta^{18}O_{shell}$ medium	0.17	2.52	0..19	-0.06 to 0.41
$\delta^{18}O_{shell}$ small vs $\delta^{18}O_{shell}$ large	0.03	0.36	0.9646	-0.22 to 0.27
$\delta^{18}O_{shell}$ large vs $\delta^{18}O_{shell}$ medium	0.15	2.41	0.2162	-0.06 to 0.36

Table 2: Results of the ANOVA test for size fractions. Comparison of the means of the three different size classes: small, medium and large.

Analysis of Variance Results					
Source	**DF**	**SS**	**MS**	**F**	**P**
Total	49	3.85	0.08	10.91	0.00013
A	2	1.22	0.61		
Error	47	2.63	0.06		

Tukey's All Pairs Comparison				
	Mean difference	|q|	P	95% CL
T. sacculifer* vs *G. ruber	0.33	5.90	0.0004	0.14 to 0.52
T. sacculifer* vs *N. dutertrei	0.03	0.44	0.9492	-0.18 to 0.24
N. dutertrei* vs *G. ruber	0.30	5.22	0.0017	0.104 to 0.504

Table 3: Results of the ANOVA test comparing the means of the three-different species vital effect corrected: *T. sacculifer*, *G. ruber* and *N. dutertrei*.

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Analysis of Variance Results					
Source	**DF**	**SS**	**MS**	**F**	**P**
Total	49	7.71	0.16	44.99	<0.0001
A	2	5.07	2.53		
Error	47	2.65	0.06		

Tukey's All Pairs Comparison				
	Mean difference	|q|	P	95% CL
T. sacculifer* vs *G. ruber	0.78	13.41	<0.0001	0.58 to 0.98
T. sacculifer* vs *N. dutertrei	0.45	7.39	<0.0001	0.24 to 0.66
N. dutertrei* vs *G. ruber	0.33	5.87	0.0004	0.14 to 0.52

Table 4: Results of the ANOVA test comparing the means of the three-different species uncorrected for vital effect: *T. sacculifer*, *G. ruber* and *N. dutertrei*.