Stable oxygen isotope composition is biased by shell calcification intensity in planktonic Foraminifera

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Abstract

Planktonic Foraminifera are widely used for environmental reconstructions through measurements of their shell’s geochemical characteristics, including its stable oxygen and carbon isotope composition. Using these parameters as unbiased proxies requires a firm knowledge of all potential confounding factors influencing foraminiferal shell geochemistry. One such parameter is the shell calcification intensity (shell weight normalized for shell size) that may influence the shell δ¹⁸O value either bioenergetically (by reducing energy available and required for equilibrium isotope fractionation during faster calcification) or kinetically (by influencing calcification depth through the shell’s density contrast with seawater). Specimens from the Globigerinoides ruber/elongatus compound from a sediment trap in the North Atlantic have been used to quantify the influence of shell calcification intensity on shell δ¹⁸O values. Shell calcification intensity was found to have a significant effect on the shell stable oxygen isotope composition in all species. Through model fitting, it is suggested that the effect size may be in a range of 1 to 2 on species, depth migration, and local oceanographic conditions). We show that the confounding effect of shell calcification intensity on stable oxygen isotope composition can be of importance, depending on the anticipated precision of the derived reconstructions. A framework is provided to quantify this effect in future studies.
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ABSTRACT

Planktonic Foraminifera are widely used for environmental reconstructions through measurements of their shell’s geochemical characteristics, including its stable oxygen and carbon isotope composition. Using these parameters as unbiased proxies requires a firm knowledge of all potential confounding factors influencing foraminiferal shell geochemistry. One such parameter is the shell calcification intensity (shell weight normalized for shell size) that may influence the shell’s δ¹⁸O value either bioenergetically (by reducing energy available and required for equilibrium isotope fractionation during faster calcification) or kinetically (by influencing calcification depth through the shell’s density contrast with seawater). Specimens from the Globigerinoides ruber/elongatus compound from a sediment trap in the North Atlantic have been used to quantify the influence of shell calcification intensity on shell’s δ¹⁸O values. Shell calcification intensity was found to have a significant effect on the shell stable oxygen isotope composition in all species. Through model fitting, it is suggested that the effect size may be in a range of 1 to 2‰ (depending on species, depth migration, and local oceanographic conditions). We show that the confounding effect of shell calcification intensity on stable oxygen isotope composition can be of importance, depending on the anticipated precision of the derived reconstructions. A framework is provided to quantify this effect in future studies.

INTRODUCTION

Planktonic Foraminifera are an abundant protist group in the world’s oceans and their calcitic shells are commonly preserved in the sediment (Schiebel, 2002; Schiebel & Hemleben, 2017). For this reason, planktonic foraminiferal shells are abundantly used for paleoenvironmental reconstructions and biomonitoring purposes (e.g., Consolaro et al., 2018; Gorbarenko & Southon, 2000; Kučera, 2007; Weinkauf et al., 2016). A major application of planktonic foraminiferal shells is the use of their geochemical compositions for reconstructions of past environmental conditions (Lea, 2002; Ravelo & Hillaire-Marcel, 2007; Rohling & Cooke, 2002; Rosenthal, 2007), which also provides the basis for climate models (e.g., Ezat et al., 2016; Ganssen & Troelstra, 1987; G. M. Ganssen et al., 2011; Groeneveld et al., 2006; Hemleben et al., 1996; Henehan et al., 2016; Ivanova, 1985; Milker et al., 2013; Steph et al., 2009).

The stable isotope composition of planktonic foraminiferal shells is usually not in equilibrium with seawater. For instance, for the shell’s stable oxygen isotope composition, a vital effect often results in a different isotopic composition compared to a composition in equilibrium with the ambient seawater (compare Spero et al., 1991; Zeebe et al., 2008). For different species, the size of this vital effect varies, necessitating species-specific correction functions to allow reliable paleoenvironmental interpretations (Carter et al., 2017; Spero, 1998; Steinke et al., 2005; Wit et al., 2013). For the application of stable oxygen isotope compositions of planktonic foraminiferal shells, for instance for paleoclimate (e.g., Ganssen & Troelstra, 1987; Milker et al., 2013) and depth habitat reconstructions (e.g., Mortyn & Charles, 2003; van Eijden, 1995), it is necessary to understand further factors that may bias the values measured within the shells. This is especially important when applying
single–shell geochemical analyses, as the geochemical variation between individual shells precipitated under comparable conditions is mostly larger than expected and beyond the analytical error of the measurement (Groeneveld et al., 2019; Killingley et al., 1981; Schillfelbin & Hills, 1984). For instance, the seasonality of species may play an important role in the observed stable isotope composition of a temporally integrated sediment sample (G. M. Ganssen et al., 2011; King & Howard, 2005), as does the intermixing of sediments due to bioturbation (Billups & Spero, 1996; Stott & Tang, 1996). The interpretation of geochemical compositions of shells may even be further complicated by intra–shell variations of the stable isotope compositions (Vetter et al., 2013) and ontogenetic changes in shell geochemistry (Rohling et al., 2004; Shackleton et al., 1985). While progress is being made in the understanding of the individual variation of the geochemical signal in planktonic Foraminifera, a larger part of the observable variation in single–shell analyses is still unaccounted for (compare Groeneveld et al., 2019, and citations therein). Incidentally, there have been few studies so far investigating to what degree the stable oxygen isotope composition of individual planktonic foraminiferal shells is influenced by their shell calcification intensity (the amount of calcite in the shell, normalized for shell size, Billups & Spero, 1995; Ezard et al., 2015).

Shell calcification intensity varies widely across planktonic foraminiferal species, and it can be measured as a environmental proxy (compare Marshall et al., 2013; Weinkauf et al., 2016). In this regard, shell calcification intensity has been mostly used as a proxy for dissolution (Broecker & Clark, 2001; Lohmann, 1995; Schiebel et al., 2007) and carbonate saturation of the seawater (Bijma et al., 2002; Bijma et al., 1999; Davis et al., 2019; de Moel et al., 2009; Osborne et al., 2016; Spero et al., 1997; Weinkauf et al., 2013). However, other studies could show that the picture is more complicated, and shell calcification intensity is indeed influenced by a variety of environmental factors (Aldridge et al., 2012; Marshall et al., 2013; Mohan et al., 2015; Weinkauf et al., 2016), which may also have an influence on the shell’s geochemical composition (e.g., water temperature, salinity).

The mechanisms of shell calcification in Foraminifera are still a matter of discussion, with seawater vacuolization, transmembrane ion transport, calcification via organic matrices, and active pH manipulation all touted as possibly valid calcification models (de Nooijer et al., 2014; de Nooijer et al., 2009; Ohno et al., 2016; Toyofuku et al., 2017). Since shell calcification in Foraminifera is so complicated and hardly understood, only comparatively little work has been performed so far to investigate its influence on the shell’s geochemical composition (e.g., Raitzsch et al., 2010; Steinhardt et al., 2015; van Dijk et al., 2017). Nevertheless, it is very feasible that calcification intensity of the shell can affect the foraminiferal shell’s geochemical composition. The first possible influence stems from the calcification process itself. Normally, organisms preferentially use lighter isotopes of elements for inclusion in their organic and inorganic structural parts when compared to inorganic calcite (De La Rocha, 2003; Gabitov et al., 2012; Kendall & Caldwell, 1998). This is because inclusion of lighter isotopes is energetically favored, since bonds are weaker with the lighter isotopes (Kendall & Caldwell, 1998). In this regard, the calcification process involves an active selection of isotopes from the seawater, which is normally considered to be the so–called vital effect (fractionation). This process itself is time– and energy consuming, especially in those calcification models that require active membrane transport of components at the time of calcification (de Nooijer et al., 2014; Toyofuku et al., 2017). A higher calcification intensity in the shell can either result from a faster calcification rate for the same amount of time, or calcification at a constant rate for a longer time–interval. Under the assumption that a higher calcification intensity in the adult foraminiferal shell was derived from a faster instead of longer calcification duration during chamber formation (Hemleben et al., 1987; Spero, 1988; Spero et al., 1991), we may assume that the faster calcification rate must increase the size of the vital effect. This scenario leaves the foraminifler with less time to perform the calcification process, necessitating a more frequent inclusion of the much more abundant lighter isotope 

\[ ^{18}\text{O} \] and thus lower \[ ^{18}\text{O} \] values of the shell when the shell’s calcification intensity increases (Norris, 1998). Such a process has for instance been observed in corals in relation to their growth rate, where faster growing parts of the skeletal structure were enriched in \[ { }^{18}\text{O} \] (McConnaughey, 1989).

The second influence stems from the effect of a heavier shell on the depth habitat of the Foraminifera. While planktonic foraminiferal species in general prefer a certain, species–specific habitat (Bé, 1977; Bé & Tolderlund, 1971; Numberger et al., 2009; Schiebel & Hemleben, 2017; Weiner et al., 2012), this depth habitat has been shown to be rather wide and reasonably flexible (compare Meillard et al., 2019; Rebotim et al., 2017). Moreover, there are signs for an ontogenetic change of the depth habitat in planktonic Foraminifera (Eggins et al., 2003; Schiebel & Hemleben, 2017; van Eijden, 1995). It can be reasonably assumed that a planktonic foraminifer with a higher calcification intensity lives in a deeper habitat than a foraminifer of a similar species with a lighter calcified shell simply due to gravitational pull and reduced buoyancy (Haenel, 1987; Weinkauf et al., in press; Zarkogradins et al., 2019). Since the stable isotope composition of the seawater varies as a function of density, which in turn is largely controlled by the salinity and temperature of water through changes in evaporation and/or freshwater contributions, this could influence the stable isotope composition of all subsequently built chambers in correlation with the shell’s calcification intensity. If higher density shells, hence, find themselves at larger depths compared to lower density shells, this could influence the \[ ^{18}\text{O} \] values of planktonic foraminiferal shells. For example, foraminiferal shells with a higher shell calcification intensity would have formed within higher–salinity or lower–temperature waters at larger water depths and, thus, show increased
We used material from the 2000 m deep sediment trap Kiel 276–25 in the North Atlantic (33° N, 22° W; Fig. 1), spanning the time between May 2005 and April 2006 with a sampling resolution of 1–2 months (Table S1). The >63 µm fraction of the dried and de-poisoned sediment trap samples were desalted using MilliQ water and then dried overnight at 50°C. Desalted samples were then split into the 63–150 µm and >150 µm size fraction using a Retsch AS 200 sieve tower (10 min. at intensity 40). Only the >150 µm size fraction was used for further analyses to avoid ontogenetic effects on calcification intensity (Peeters et al., 1999). All planktonic Foraminifera from all samples were separated from the residue. Planktonic Foraminifera for shell calcification analyses were picked from representative samples of those concentrates, split with an ASC Scientific MS-1 microsplitter, such that approximately 100 specimens per sample were available for analyses. In samples with fewer specimens, 100% of the sample was used (Table S2).

All specimens for analyses were cleaned with 2% ethanol and brushes and then rinsed in distilled water and on wet filter paper. They were then transferred into 10-hole cardboard slides and left to dry at room temperature for at least 24 hours.

Choice of species

The species were chosen to fulfill certain criteria. (1) They had to have a relatively restrictive depth habitat, so that our environmental reconstructions were effective in eliminating confounding factors, but still large enough to observe habitat depth changes. (2) They needed to be abundant enough for a reliable analysis. (3) They had to be species which are often used in geochemical analyses to maximize the practical value of the study and stay comparable with the literature.

We thus selected the three species Globigerinoides ruber (pink), Globigerinoides ruber (white), and Globigerinoides elongatus (Fig. 2). They all have photosymbionts (Schiebel & Hemleben, 2017) and are, thus, restricted to the photic zone, as is also confirmed by in situ studies (Meilland et al., 2019; Rebotim et al., 2017; Steinhardt et al., 2015). They are extensively used in geochemical analyses and especially G. ruber (white) and G. elongatus were studied in detail concerning their differences in geochemical shell composition (Carter et al., 2017; Kawahata, 2005; Numberger et al., 2009; Steinke et al., 2005; Wang, 2000). We here follow the suggestions by Aurahs et al. (2011) to distinguish G. ruber (white) and G. elongatus: Globigerinoides elongatus has a flat last and penultimate chamber and especially the last chamber is very asymmetrical, while these chambers are inflated and symmetrical in G. ruber (white). The separation into G. ruber (white) and G. elongatus was...
entirely done by the same scientists (principal author) to avoid any influence of competing species concepts.

Calcification intensity analysis

All specimens where weighed individually on a Mettler Toledo UMX 2 microbalance (nominal precision 0.1 µg). The empty weighing boats (Elemental Microanalysis D5007) were first weighed ten times to reliably determine their weight. Afterwards, individual Foraminifera were transferred into the weighing boats and weighed four times each, and the difference between the empty and filled weighing boat was noted as the weight (W) of the foraminifer. The replication of weighing per individual allows to determine the measurement error of the weighing procedure.

The same specimens were then photographed in apertural standard view at 27.5× magnification on a Leica M420 light microscope using a Leica DFC420 camera. The cross-sectional area (A) of each foraminifer was extracted in Fiji (Schindelin et al., 2012) running ImageJ v. 1.52o (Schneider et al., 2012), and the calcification intensity $\rho_A$ sensu Marshall et al. (2013) was calculated as $\rho_A = \frac{W}{A}$ (compare Fig. S1).

Stable isotope analyses

Approximately 50 specimens per species for stable isotope analysis were selected using a stratified random sampling approach. For this, the sample was divided into 4 strata based on the range of observed specimen calcification intensity, and an equal number of specimens was randomly selected from each stratum. Thus, it could be made sure that all $\rho_A$ classes were equally represented in the analyses. All geochemical measurements were performed at the Faculty of Geosciences and Environment of the University of Lausanne.

Selected specimens were individually cleaned via sonication (3 min.) in Krantz cells filled with tap water to remove any potential contaminants from the handling during the calcification intensity analysis. Specimens were then transferred into glass vials, closed with screw-on septum caps, and then analyzed for their $\delta^{13}C$
and δ18O values on a Gas Bench II He-carrier gas system connected to a ThermoFisher DeltaPlus XL mass spectrometer (method by Spötl & Vennemann, 2003, adapted for small vials of 4 ml volume; acidifying with four drops of orthophosphoric acid of a specific gravity of 1.90, and measuring only four instead of ten peaks for each sample). Carrara marble was used as a standard and was measured alongside the foraminiferal shells every six samples and additionally two times at the start and at the end of each sequence. Standards and samples had the same weights and, hence, yields for the carbonate. All values are expressed in the familiar δ-notation and normalized against NBS-19 on the VPDB-scale. Repeated measurements for the standards after a size correction are within 0.1‰ for both oxygen and carbon isotope compositions.

 Environmental setting

The sediment trap is located close to the Azores Front, which separates the temperate North Atlantic water from the North Atlantic subtropical gyre and is influenced by both water regimes (Figs. 1 and 3; Fründt & Waniek, 2012). Environmental data were calculated as averages for the respective sample duration and the estimated catchment area of the sediment trap (based on J. Waniek et al., 2000; J. J. Waniek et al., 2005) (Table S1). Sea surface temperature (SST) is based on the NOAA Optimum Interpolation (OI) Sea Surface Temperature (SST) V2 provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA (https://www.esrl.noaa.gov/psd/; Reynolds et al., 2002). Sea surface salinity (SSS) was calculated from the EN4 quality controlled ocean data (v. EN4.20; Good et al., 2013). The carbonate system of the seawater was estimated using the MS Excel program CO2Sys v. 2.1 (Lewis et al., 1998) with K1 and K2 constants from Mehrbach et al. (1973) (refit by Dickson & Millero, 1987) and KSO4 constant after Dickson (1990).

 Data analysis

All data analyses were performed in R v. 3.6.2 (R Core Team, 2019) and interpreted following Murthaugh (2014). Differences between parameters across species were tested using pairwise Mann–Whitney U tests (Mann & Whitney, 1947) with p-values corrected for the false discovery rate after Benjamini and Yekutieli (2001). The data for regression analyses were cleaned from potential outliers using bagplots of δ18O against calcification intensity (Rousseeuw et al., 1999) as implemented in the R-package "aplpack" v. 1.3.3. Problems with multicollinearity in the data were eliminated following suggestions by Dormann et al. (2013): (1) The presence of a considerable degree of multicollinearity in the data was tested based on the condition number (Dormann et al., 2013). (2) Multicollinearity-clusters in the predictor variables were identified using principal component analysis (PCA; Booth et al., 1994) and cluster analysis on Hoeffding’s (1948) D-similarity index (Kauffmann & Rousseeuw, 2009). (3) The ranking of collinear parameters was established based on the deviance in bivariate regressions with δ18O values. (4) Sequential regression (Graham, 2003) was used to calculate the residuals of lower-ranked parameters and we used these as new parameters for the ensuing analyses (Dormann et al., 2013). We further applied general additive models (Friedman & Stuetzle, 1981) to identify all factors influencing the δ18O values of the foraminiferal shells in the R-package “gam” v. 1.16.1, and tested the significance of the influence of shell calcification intensity via analysis of variances (ANOVA; Fisher, 1919) of the additive model against a null-model without calcification intensity. When the shell calcification intensity had a significant effect when accounting for all other parameters, linear and non-linear regression was used to fit different models to the data. Because most of the equations of the fitted models can only describe relationships in the first quadrant (to avoid e.g., division by zero), 10% had been added to all δ18O values to transform them into positive values for this step. The corrected Akaike information criterion (AICc; Akaike, 1974) in combination with Akaike weights (Wagenmakers & Farrell, 2004) was used to determine the best fitting model from a set of mechanistically feasible candidate models and estimate the effect size. The relationship between δ18O and δ13C was investigated using a Deming regression (Deming, 1943) that took the associated measurement error in both values into account, using the R-package “deming” v. 1.4.

 RESULTS

We analyzed the shell calcification intensity and shell geochemical composition of 136 specimens of the Globigerinoides ruber/elongatus compound from four samples covering an entire year from sediment trap Kiel 276-25 (Fig. S2, Table S1). In total, 42 specimens of Globigerinoides ruber (pink), 42 specimens of Globigerinoides ruber (white), and 52 specimens of Globigerinoides elongatus were successfully analyzed. The results have been summarized in Fig. 4 and Table S3.

In terms of raw shell weight, we note that specimens of G. ruber (pink) are on average more than twice as heavy as specimens of G. ruber (white) and more than 25% heavier than G. elongatus specimens. The difference between all species is moderately significant at p < 0.02, indicating a general inherent difference in net shell weight. A different picture emerges when examining the shell calcification intensities. Globigerinoides elongatus is most heavily calcified, followed by G. ruber (pink) and G. ruber (white). However, these differences are all very small and insignificant at p > 0.3, implying a comparable size-weight ratio between the species, which suits well with the fact that they are closely related and inhabit roughly the same portion of the water column, which requires a comparable density contrast.
For the stable isotope composition, we also see considerable differences between the species. The δ\(^{13}\)C values of G. ruber (pink) are positive, while they are negative for the other species, with G. ruber (white) having the lowest values. The difference in average δ\(^{13}\)C values is moderately significant for all pairwise comparisons (\(p < 0.02\)). For the stable oxygen isotopes, we see another signal. Values are highest for G. elongatus and lowest for G. ruber (pink), but the differences between neither G. ruber (pink) and G. ruber (white) (\(p = 0.131\)) nor G. ruber (white) and G. elongatus (\(p = 1.000\)) are significant. Only between G. elongatus and G. ruber (pink) do differences in δ\(^{18}\)O values of the shell accumulate to a convincingly significant signal (\(p = 0.002\)).

\[
\delta^{18}O = \ell(T) + \ell(S) + \ell(\sigma_P) + \ell(\rho_A) + \epsilon
\]

with \(T\) as sea surface temperature, \(S\) as sea surface salinity, \(\sigma_P\) as photosynthetic activity, \(\rho_A\) as shell calcification intensity, \(\epsilon\) as error term, and \(\ell\) indicating a LOESS smooth.

We applied general additive models to evaluate the influence of shell calcification intensity on the stable oxygen isotope composition of shells within the Globigerinoides ruber/elongatus compound. To disentangle this influence from the effects of water temperature, salinity, and symbiont-photosynthetic activity (approximated by shell δ\(^{13}\)C values), which all influence the δ\(^{18}\)O values of foraminiferal shells, we used the model described in Eq. (1).
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G. elongatus G. ruber

5 10 15 20 25
Shell weight (µg)

G. elongatus G. ruber

0.00010 0.00015 0.00020 0.00025 0.00030 0.00035
Calcification intensity (µg µm$^{-2}$)

G. elongatus G. ruber

−2.0 −1.5 −1.0 −0.5 0.0 0.5 1.0
δ$^{13}$C VPDB (‰)

G. elongatus G. ruber

−2.0 −1.5 −1.0 −0.5 0.0 0.5 1.0
δ$^{18}$O VPDB (‰)

Fig. 4: Comparison of shell weight (a), shell calcification intensity (b), and shell δ$^{13}$C (c) and δ$^{18}$O (d) values of shells of Globigerinoides ruber (pink), Globigerinoides ruber (white), and Globigerinoides elongatus from sediment trap Kiel 276-25. Thick lines indicate median, boxes cover interquartile range (IQR), whiskers extend to 1.5 × IQR, outliers are marked by black diamonds. Small letters above the boxes denote groups according to pairwise Mann–Whitney $U$ tests.

presence of multicollinearity between any of the parameters by calculating the condition number of the correlation matrix as $\kappa = 11.11$, far in excess of the suggested threshold of $\kappa \geq 5$ that would imply the presence of collinearity. An ensuing PCA, where elements which show a loading $L > 0.3$ on the first principal component (PC) are supposed to be collinear, suggests a noteworthy collinearity between temperature ($L_{PC1} = 0.68$), salinity ($L_{PC1} = 0.65$), and photosynthetic activity ($L_{PC1} = 0.33$). A cluster analysis (complete linkage) on the Hoeffding $D$-similarity index supports this collinearity, with only shell calcification intensity showing a similarity below 0 with the other parameters (Fig. S3).

To eliminate the collinearity between temperature, salinity, and photosynthetic activity before calculation of the general additive models, we used general linear regression to regress collinear parameters against δ$^{18}$O as dependent variable. Through the deviance $d$ of these regressions, we could establish the relative importance of the parameters on the δ$^{18}$O of the foraminiferal shells as (1) temperature ($d = 45.36$), (2) photosynthetic activity ($d = 42.81$), and (3) salinity ($d = 42.35$). Through sequential regression using general linear models, we then calculated the residuals for photosynthetic activity and salinity for use in the additive models instead of the raw values of these parameters. Based on these corrections for multicollinearity, the additive models we calculated (based on Eq. 1) are described in Eq. (2).

$$\delta^{18}O = \ell(T) + \ell\left(e_{T+AP}(S)\right) + \ell\left(e_{T}(AP)\right) + \ell\left(P_{A}\right) + \epsilon$$

with $e$ as residuals and other parameters as for Eq. (1).
Results of the additive models according to Eq. (2) are summarized in Table 1. For *G. ruber* (pink), no outliers were detected, but we encountered the problem that because one sample did not contain any specimens, the data set was not suitable for an additive model approach because it had only three distinct temperature and salinity values. We thus limited the analyses to the photosynthetic activity and calcification intensity parameters in this species. The model suggests an influence of shell calcification intensity on shell $\delta^{18}O$ values and a significant increase in model fit when adding shell calcification intensity in comparison with the model that ignores it ($\rho = 0.031$). Ultimately, we can use the results from the other species to get a clearer picture of the influence of shell calcification intensity on the stable oxygen isotope composition of the foraminiferal shells considering all other parameters, even though this was impossible in *G. ruber* (pink). In *G. ruber* (white), no outliers were detected. Using an additive model, we could identify that all included parameters have a moderately to convincingly significant influence on the shell $\delta^{18}O$ values. An ANOVA against the null-model confirms the significant increase in model fit when shell calcification intensity is included at $\rho < 0.001$. In *G. elongatus*, two specimens (Specimen 77 from Sample 3 and Specimen 88 from Sample 5) were identified as outliers and removed from further analyses. The additive model confirms a convincingly significant influence of temperature, photosynthetic activity, and calcification intensity, but not salinity, on the stable oxygen isotope composition of the shells. Shell calcification intensity improves the model significantly in comparison to the null-model ($\rho = 0.049$). In all species analyzed here, we thus find a significant influence of shell calcification intensity on the shell $\delta^{18}O$ values when accounting for all other relevant parameters.

**DISCUSSION**

The average $\delta^{18}O$ values are negative in *Globigerinoides ruber* (pink), *Globigerinoides ruber* (white), and *Globigerinoides elongatus* in our study, and are compatible with a typical foraminiferal vital effect and with a preference for lighter $^{18}O$ during calcification in all species. The general trend in all species is an increase in $\delta^{18}O$ (toward zero) with increasing shell calcification intensity. This trend indicates that the influence of shell calcification intensity on $\delta^{18}O$ is linked to depth habitat changes when the density contrast between the shell and ambient seawater changes. If calcification intensity influences $\delta^{18}O$ values through a modification of living depths via changing density contrasts with the surrounding seawater (Weinkauf et al., in press; Zarkogiannis et al., 2019), we would expect lighter individuals that live in shallower depths and thus warmer water (especially in summer, we could expect temperature changes of ca. 6 °C within the upper 100 m of the water column; compare Fig. 3), to have lower $\delta^{18}O$ values; this is indeed noted by the present data. In contrast, if faster calcification rates would limit the time or energy available for fractionation, heavier calcified individuals would show reduced $\delta^{18}O$ values because of unselected uptake of the more abundant $^{16}O$ (Norris, 1998).

To better understand the observed relationship between shell calcification intensity and shell $\delta^{18}O$ values and estimate the effect size, we fitted linear and nonlinear regressions to the data (Bolker, 2008) and determined, which would best describe the observations using the corrected Akaike information criterion ($\text{AIC}_C$).

We tried the following regression functions: (1) A Michaelis–Menten (Michaelis & Menten, 1913) function and (2) a power-law function, which through their bioenergetic applicability would both be suitable candidate functions (Raitzsch et al., 2010; Russell et al., 2004). (3) A monomolecular function and (4) a second-degree polynomial function, which are functionally suitable for fractionation processes during calcification (Groeneveld et al., 2018; Pearson, 2012; Steinhardt et al., 2015). The results of this model assessment are summarized in Fig. 5 and Table 2. In *G. ruber* (pink), the data are best described by a polynomial function and second-best by a monomolecular function. The estimated effect size using the two best-fitting models is 0.63–1.01‰. A similar signal is obtained for *G. ruber* (white), where a monomolecular function describes the data much better than the second-best model (Michaelis–Menten function; normalized probability: 0.587). The effect size in *G. ruber* (white) is approximately in the range of 1.37–1.42‰. In *G. elongatus*, the power-law and Michaelis–Menten models outperform both alternative models, indicating a smaller effect size of ca. 0.56‰.

We consider the measured shell geochemical composition in our data largely free from constraining influences. It is known that photosynthetic activity influences the $\delta^{18}O$ value measured in foraminiferal shells (Ezard et al., 2015; Spero, 1998; Spero & Lea, 1993), but $\delta^{13}C$ is directly scaled to photosynthetic activity or bioproductivity, and hence also to depth (e.g., Norris, 1998; Spero, 1993; Spero et al., 1991). Therefore, by including shell $\delta^{13}C$ values in our general additive models, we can test for the effect of calcification intensity on shell $\delta^{18}O$ values, regardless. A shell size effect on $\delta^{13}C$ has been noted, which does not exist for $\delta^{18}O$ to any noteworthy extent in the species used here (Ezard et al., 2015; Franco-Fraguas et al., 2011; Norris, 1998; Shackleton et al., 1985), meaning that our analyses are robust against the shell size range used in this study. Carbonate ion concentration can have an effect on stable isotope compositions of planktonic Foraminifera, especially $\delta^{13}C$ values (Spero, 1998), but the sampling region is considerably stable concerning its carbonate system. We used data from the ESTOC time series (González-Dávila, 2016a, 2016b), which is situated close to sediment trap Kiel 276, and the MS Excel program CO2Sqs to estimate the carbonate system parameters. The maximum annual range of
Tab. 1: Results of an additive model analysis of stable oxygen isotope composition against temperature ($T$), salinity ($S$), photosynthetic activity ($\rho_P$), and shell calcification intensity ($\rho_f$) in foraminiferal shells from sediment trap Kiel 276-25.

| Parameter                       | Mean squares | $F$-value | $p$-value | Nonparametric terms |
|---------------------------------|--------------|-----------|-----------|---------------------|
| **Globigerinoides ruber (pink)**|              |           |           |                     |
| $\alpha_P$                      | 4.10         | 44.58     | <0.001    | 3.48                |
| $\rho_f$                        | 0.25         | 2.69      | 0.111     | 5.03                |
| **Globigerinoides ruber (white)**|              |           |           |                     |
| $T$                             | 61.36        | 371.03    | <0.001    | 20.90               |
| $S$                             | 1.59         | 9.61      | 0.004     | 21.00               |
| $\alpha_P$                      | 26.83        | 162.22    | <0.001    | 0.81                |
| $\rho_f$                        | 5.66         | 34.25     | <0.001    | 2.12                |
| **Globigerinoides elongatus**    |              |           |           |                     |
| $T$                             | 48.59        | 536.71    | <0.001    | 200.39              |
| $S$                             | 0.00         | 0.00      | 0.989     | 97.95               |
| $\alpha_P$                      | 1.57         | 17.34     | <0.001    | 1.84                |
| $\rho_f$                        | 0.75         | 8.32      | 0.007     | 0.98                |

Tab. 2: Results from a fitting of four model functions to the influence of shell calcification intensity on shell $\delta^{18}O$ in foraminiferal shells from sediment trap Kiel 276-25. The corrected Akaike information criterion ($AIC_c$; Akaike, 1974) and model weights (Wagenmakers & Farrell, 2004) are reported.

| Parameter                       | Michaelis–Menten function | Power-Law function | Monomolecular function | 2nd-degree polynomial function |
|---------------------------------|----------------------------|--------------------|------------------------|--------------------------------|
| **Globigerinoides ruber (pink)**|                            |                    |                        |                                |
| $AIC_c$                         | 55.175                     | 56.953             | 52.577                 | 51.526                         |
| $\Delta AIC_c$                   | 3.649                      | 5.427              | 1.051                  | 0.000                          |
| Weight                          | 0.089                      | 0.036              | 0.325                  | 0.550                          |
| **Globigerinoides ruber (white)**|                            |                    |                        |                                |
| $AIC_c$                         | 94.469                     | 95.207             | 93.767                 | 96.263                         |
| $\Delta AIC_c$                   | 0.702                      | 1.440              | 0.000                  | 2.496                          |
| Weight                          | 0.284                      | 0.196              | 0.404                  | 0.116                          |
| **Globigerinoides elongatus**    |                            |                    |                        |                                |
| $AIC_c$                         | 41.277                     | 41.093             | 42.097                 | 43.340                         |
| $\Delta AIC_c$                   | 0.184                      | 0.000              | 1.003                  | 2.247                          |
| Weight                          | 0.321                      | 0.352              | 0.213                  | 0.114                          |

CO$_3$$^2$– in surface waters in the region is $<50$ µmol kg$^{-1}$, and the average range is as small as $<10$ µmol kg$^{-1}$ throughout spring–autumn (Table S4), ruling out a large effect of this parameter. We therefore believe that a better interpretation of the data is required.

One possible solution to explain the observations would be dissolution of the shells (Broecker & Clark, 2001; Lehmann, 1995; Schiebel et al., 2007), as selective dissolution of poorly crystallized, small crystallites of calcite with a particular stable oxygen isotope composition could result in a spurious $\delta^{18}O$–calcification intensity-correlation. This is unlikely, however, for the following reasons. (1) We used the same stratified random sampling applied to pick the specimens for isotope analyses to choose a second random sample per species to be investigated under the scanning electron microscope. The vast majority of the shells shows good to excellent preservation, with minor re-crystallization observable in only a small number of shells (Figs. S4–S6). (2) This is supported by a reconstruction of the carbonate system using the ESTOC time series (González-Dávila, 2016a, 2016b). Throughout the entire sampling interval, the calcite saturation state at the surface is $>4$, and even at the sediment trap depth of 2000 m it never decreases below 1.38 at any time (Table S4). The latter value is near the lower boundary below which dissolution starts to affect shell geochemistry (Dekens et al., 2002; Regenberg et al., 2006), but not yet problematic. (3) We further note that, should dissolution play a major role, we would expect to see the inverse effect. This is because calcite that was precipitated in warmer waters has a higher Mg/Ca ratio, and is generally dissolved more readily than calcite precipitated in colder waters (de Villiers et al., 2002; Rosenthal et al., 2003). Since calcite that was formed in warmer water would also have lower
Fig. 5: Models for the shell $\delta^{18}O$ values depending on the shell calcification intensity in the planktonic foraminifers (a) *Globigerinoides ruber* (pink), (b) *Globigerinoides ruber* (white), and (c) *Globigerinoides elongatus* from sediment trap Kiel 276-25. (d) Relationship between shell $\delta^{13}C$ and $\delta^{18}O$ in the same samples. Error bars indicate standard error of the measurements.

$\delta^{18}O$ values, this process of selective dissolution would increase the $\delta^{18}O$ values of the lighter shells, such that the pattern we observe here would have been even more pronounced if such a selective dissolution would have taken place.

The other possibility is an environmental effect. On the one hand, this could for instance be a salinity effect. Although salinity was determined as least important and non-significant parameter, and is strongly collinear with temperature, this is only true for the remote and averaged environmental data available here. It was shown that such data often underestimate the true short-term variation in oceanic settings (Laer Yet Huybers, 2014a, 2014b). *Globigerinoides ruber* is very resistant against salinity changes (Bijma et al., 1990) and planktonic Foraminifera can build new chambers within hours (Bé et al., 1979; Spero, 1988), which would make it theoretically feasible that the local salinity could have changed sufficiently on smaller time-scales (e.g., via north–south fluctuation of the Azores front; Fründt & Waniek, 2012) without influencing chamber formation in the Foraminifera, thus explaining part of the measured range of compositions. However, this is unlikely to be responsible for the majority of the correlation due to the inconceivable amplitude of salinity changes in an open-ocean setting this assumption would require. In fact, even through fluctuation of the Azores front, salinities beyond 36.2 are practically never observed in this oceanographic setting (Fründt & Waniek, 2012; Pérez et al., 2003). Temperature changes due to vertical migration, on the other hand, could explain a major part of the measured differences in isotopic compositions for shells with different calcification intensities (Rebotim et al., 2019). The depth habitat of species in the *Globigerinoides ruber/elongatus* compound is generally shallow, partly due to its dependence on sunlight for photosynthesis, but both living- and calcification-depths were shown to be reasonably variable...
within the uppermost 50–100 m of the water column (Meilland et al., 2019; Rebottin et al., 2017; Steinhardt et al., 2015). Within the studied area, the mixed layer covers this depth in winter, with an average temperature change of only 0.3 °C over the top 100 m. During summer, however, the average temperature variation across the first 100 m is ca. 6 °C, and thus large enough to explain perhaps 1.5–2‰ of the observed variation in shell δ¹⁸O (compare Fig. 3). Even in winter, changes beyond the recorded mean values can occur due to the fluctuation of the Azores front across the sediment trap mooring (Fründt & Wanike, 2012).

Interestingly, δ¹³C values can be interpreted as a water depth indicator, especially pronounced in photosymbiont-bearing Foraminifera (Spero & Williams, 1988). For this reason, one would expect to note a positive correlation between δ¹³C and δ¹⁸O in such foraminiferal assemblages, where the recorded temperature changes are at least partly related to depth migration (Norris, 1998, Spero & Williams, 1988). We see a pronounced pattern of this type in G. ruber (pink) and G. ruber (white), but a much weaker signal in G. elongatus for our dataset (Fig. 5d). Coincidentally, G. elongatus is also the species that shows the smallest effect size of shell calcification intensity on shell δ¹⁸O values. It was shown by Steinke et al. (2005) that G. elongatus lives generally deeper than G. ruber, but because the species is still dependent on light for its photosymbionts this implies that extensive depth migration is less of a possibility for this species, which could explain why a more stable depth habitat in G. elongatus is compatible with our data. We hypothesize here that the measured compositions in all species are the result of a depth migration-related temperature change (directly scaled to shell calcification intensity via density contrast with the seawater). We further hypothesize that G. elongatus shows the smallest effect size due to its limited depth migration. If this interpretation is true, then shell calcification intensity can be a viable proxy to reduce the bias in geochemical shell measurements of planktonic Foraminifera that is associated with habitat depth. Our study can consequently be used in the implementation of bias-modelling of climate archive proxies as for instance through “Sedproxy” (Dolman & Laepple, 2018), and can help to further improve the reliability of climate reconstructions (Dolman et al., 2020). Such an interpretation is also in line with earlier observations that larger shells within some species have higher δ¹³C values (e.g., Spero & Lea, 1993). Larger shells contain more cytoplasm that has a negative density contrast to the surrounding seawater. Unless the foraminifer increases it’s relative shell thickness (to keep the shell calcification intensity constant), it will be subject to greater buoyant forces, reducing its depth habitat as shown by the higher δ¹³C values of its shell. This is also implied by observations in Zarkogiannis et al. (2019), where it is shown that larger shells tend to be more buoyant because their weight does not increase at the same rate as their volume if the shell thickness remains constant.

Further studies are suggested in this field, ideally involving controlled laboratory experiments, especially regarding if this effect also exists for asymbiotic Foraminifera – as is implied by Billups and Spero (1995) at least for small specimens – or species which tend to develop thick crusts (Schiebel & Hemleben, 2017, e.g., Globoconella inflata, Globorotalia crassidentata, Globorotalia truncatulinoides). If our hypothesis is true, the effect of shell calcification intensity on the stable oxygen isotope composition of foraminiferal shells through depth habitat changes is rather variable between locations and species. This means that the effect on temperature reconstructions can be small enough that for most purposes a correction for shell calcification intensity does not need to be applied, but a bias of around 1°C seems feasible under certain oceanographic conditions. Such effects may be important for some studies (e.g., Dolman et al., 2020), especially in regions or species where shell calcification intensity varies more strongly within a species and in environments with strong vertical changes in the ambient seawater environment. We presented in this study a framework to deal with this problem in the future, by quantifying shell calcification intensity of specimens intended for geochemical analyses and quantifying its effect size for data correction. Since especially the portion of the influence that is caused by depth migration will be dependent on the investigated species (theoretical range of depth habitat), observed range of calcification intensity (practical range of depth habitat), and the local oceanography, no generally applicable corrections can be suggested here. We thus suggest an implementation of this factor in bias-modelling protocols in the future (e.g., Dolman & Laepple, 2018).

**CONCLUSIONS**

Our analyses of foraminiferal species of the *Globigerinoides* *ruber/elongatus* compound imply that shell calcification intensity has an impact on shell δ¹⁸O values and therefore has the potential to be a confounding factor in environmental reconstructions derived from stable oxygen isotope measurements of planktonic foraminiferal shells.

Our data suggest that the observed effect is mainly an environmental effect, where heavier calcified shells would occupy a deeper habitat of the water column and are thus exposed to lower temperatures of their ambient seawater. The effect size will depend on the species (range of depth habitat; compare Rebottin et al., 2019), the range of shell calcification intensity, and the local oceanography.

We suggest using shell calcification intensity studies to evaluate and eliminate this effect on geochemical analyses if high-precision reconstructions are needed, but argue that the effect is probably small enough to not interfere with the majority of practical applications. We suggest further studies in this field and an implementation of this bias in standard bias-modelling protocols.
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Electronic supplements to: “Stable oxygen isotope composition is biased by shell calcification intensity in planktonic Foraminifera”

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1 Material and methods

| Sample | Start date | End date | Sampling dur. (d) | SST (°C) | SSS |
|--------|------------|----------|-------------------|----------|-----|
| 1      | 2005-05-01 | 2005-07-01 | 61                | 20.95    | 36.63 |
| 3      | 2005-09-01 | 2005-11-01 | 61                | 22.93    | 36.76 |
| 5      | 2006-01-01 | 2006-02-01 | 31                | 18.25    | 36.65 |
| 7      | 2006-03-01 | 2006-04-01 | 31                | 18.23    | 36.58 |

SST: Sea surface temperature after Reynolds et al. (2002); SSS: Sea surface salinity after Good et al. (2013)

| Species | Sample 1 | Sample 3 | Sample 5 | Sample 7 |
|---------|----------|----------|----------|----------|
| Globigerinoides ruber (pink) calci. | 27       | 55       | 2        | 0        |
| Globigerinoides ruber (pink) geochem. | 19       | 21       | 2        | 0        |
| Globigerinoides ruber (white) calci. | 60       | 92       | 70       | 34       |
| Globigerinoides ruber (white) geochem. | 11       | 7        | 12       | 12       |
| Globigerinoides elongatus calci. | 38       | 75       | 34       | 41       |
| Globigerinoides elongatus geochem. | 13       | 12       | 13       | 14       |
Fig. S1. Flowchart of size measurements of planktonic foraminiferal shells for shell calcification intensity analyses. The contrast in the raw image (a) with a planktonic foraminifer in apertural standard view is increased and the image is converted into a black-and-white threshold image (b). Within this image, the shell is automatically traced and its size measured as cross-sectional area (c).
2 Results

**Fig. S2.** Sea surface temperature Reynolds et al. (2002, a) and sea surface salinity Good et al. (2013, b) in the catchment area of sediment trap Kiel 276-25 during the sampling period. The range of values across the catchment area is indicated as shaded area, the sampling intervals are indicated as grey rectangles.

**Table S3.** Measured mean foraminiferal shell parameters of three species of planktonic Foraminifera from sediment trap Kiel 276-25.

| Species          | Diam. (μm) | Weight (μg) | $\rho_A$ ($1 \times 10^4$ μg μm$^{-2}$) | $\delta^{13}$C (‰) | $\delta^{18}$O (‰) |
|------------------|------------|-------------|---------------------------------------|---------------------|---------------------|
| G. ruber (pink)  | 334.2      | 10.15       | 1.31                                  | 0.334               | -0.533              |
| G. ruber (white) | 240.8      | 4.90        | 1.26                                  | -0.588              | -0.363              |
| G. elongatus     | 273.5      | 6.89        | 1.43                                  | -0.215              | -0.206              |

Diam: Shell Feret diameter; $\rho_A$: Shell calcification intensity; stable isotopes measured on the Vienna Pee Dee Belemnite scale normalized against NBS-19
Fig. S3. Complete linkage clustering on the Hoeffding $D$-similarity Hoeffding (1948) of sea surface temperature Reynolds et al. (2002), sea surface salinity Good et al. (2013), photosynthetic activity (approximated by shell $\delta^{13}C$), and shell calcification intensity of planktonic Foraminifera from sediment trap Kiel 276-25. A similarity value of 0 (values >0 imply collinearity) is indicated by the dashed grey line.

3 Discussion

Table S4. Estimated carbonate system parameters of the ambient sea water in the region of sediment trap Kiel 276-25, based on data from the ESTOC time series (González-Dávila, 2016a, 2016b).

| Date       | Depth (m) | $T$ ($^\circ$C) | $S$ | $TA$ (μmol kg$^{-1}$) | pH   | $CO_3^{2-}$ (μmol kg$^{-1}$) | $\Omega_{Ca}$ |
|------------|-----------|----------------|-----|----------------------|------|----------------------------|---------------|
| 2005-03-19 | 10.0      | 18.0           | 36.7| 2401.90              | 8.00 | 171.86                     | 4.05          |
| 2005-03-19 | 1976.9    | 4.5            | 35.1| 2340.99              | 7.75 | 60.93                      | 1.40          |
| 2005-04-13 | 0.3       | 19.1           | 36.7| 2402.38              | 8.01 | 181.67                     | 4.29          |
| 2005-04-13 | 200.1     | 16.9           | 36.4| 2392.03              | 7.94 | 146.00                     | 3.44          |
| 2005-09-28 | 5.0       | 23.9           | 36.9| 2418.41              | 8.03 | 220.98                     | 5.22          |
| 2005-09-28 | 199.9     | 16.6           | 36.4| 2387.05              | 7.92 | 140.08                     | 3.30          |
| 2005-11-22 | 11.6      | 22.0           | 36.9| 2412.60              | 8.03 | 207.44                     | 4.90          |
| 2005-11-22 | 1800.0    | 4.4            | 35.1| 2343.95              | 7.76 | 61.94                      | 1.43          |
| 2006-03-04 | 12.7      | 17.9           | 36.7| 2405.59              | 8.00 | 172.41                     | 4.06          |
| 2006-03-04 | 2002.2    | 4.4            | 35.1| 2342.17              | 7.74 | 60.17                      | 1.38          |

$T$: Water temperature; $S$: Water salinity; $TA$: Total alkalinity; pH measured on the total scale; $CO_3^{2-}$: Carbonate concentration; $\Omega_{Ca}$: Calcite saturation state
4 Scanning electron micrographs

Fig. S4. Scanning electron micrographs of randomly selected specimens of *Globigerinoides ruber* (pink) from sediment trap Kiel 276-25.
**Fig. S4.** Scanning electron micrographs of randomly selected specimens of *Globigerinoides ruber* (pink) from sediment trap Kiel 276-25 (continued).
Fig. S4. Scanning electron micrographs of randomly selected specimens of *Globigerinoides ruber* (pink) from sediment trap Kiel 276-25 (continued).
**Fig. S4.** Scanning electron micrographs of randomly selected specimens of *Globigerinoides ruber* (pink) from sediment trap Kiel 276-25 (continued).
**Fig. S5.** Scanning electron micrographs of randomly selected specimens of *Globigerinoides ruber* (white) from sediment trap Kiel 276-25.
Fig. S5. Scanning electron micrographs of randomly selected specimens of *Globigerinoides ruber* (white) from sediment trap Kiel 276-25 (continued).
Fig. S5. Scanning electron micrographs of randomly selected specimens of Globigerinoides ruber (white) from sediment trap Kiel 276-25 (continued).
Fig. S5. Scanning electron micrographs of randomly selected specimens of *Globigerinoides ruber* (white) from sediment trap Kiel 276-25 (continued).
Fig. S6. Scanning electron micrographs of randomly selected specimens of *Globigerinoides elongatus* from sediment trap Kiel 276-25.
Fig. S6. Scanning electron micrographs of randomly selected specimens of *Globigerinoides elongatus* from sediment trap Kiel 276-25 (continued).
Fig. S6. Scanning electron micrographs of randomly selected specimens of *Globigerinoides elongatus* from sediment trap Kiel 276-25 (continued).
Fig. S6. Scanning electron micrographs of randomly selected specimens of *Globigerinoides elongatus* from sediment trap Kiel 276-25 (continued).

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