

We kindly thank Brett Metcalfe for the positive feedback, very constructive and thoughtful comments, which greatly helped to improve our manuscript. Below you find our responses to each point which was addressed and how we incorporated all suggestions in our revised manuscript. Referee comments are written in italics and the respective answers are given in normal font in blue.

Yours sincerely

Anna Jentzen and on behalf of all co-authors

## Answers to referee 2: *B. Metcalfe*

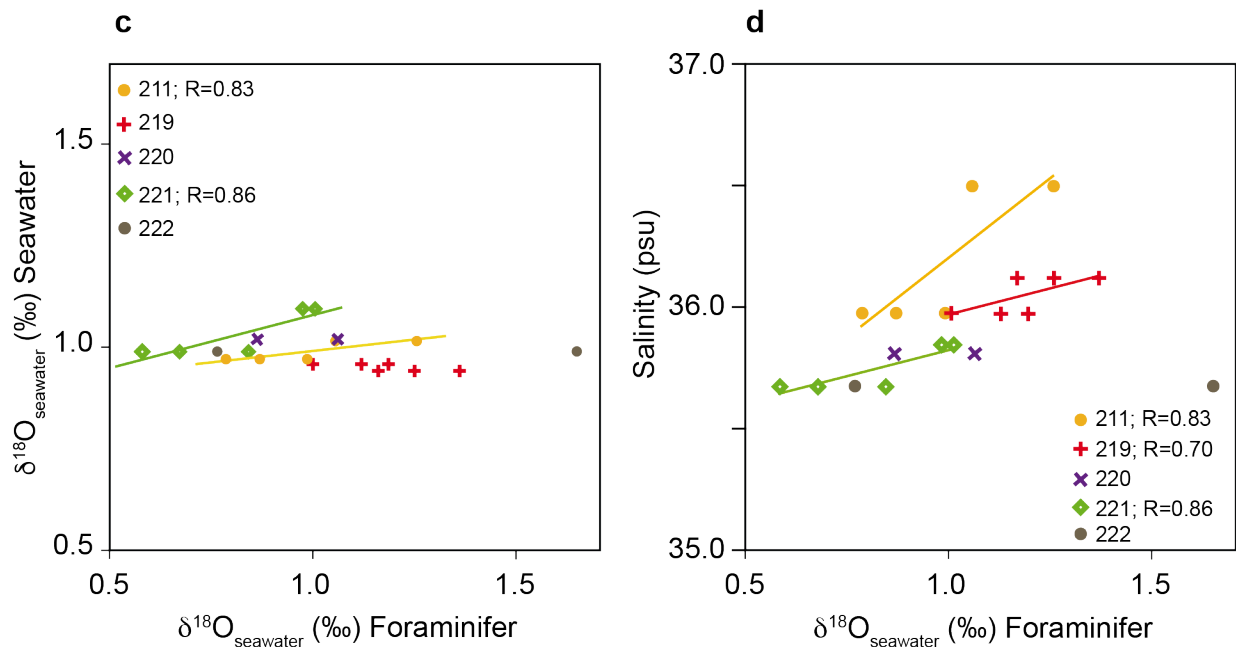
### Major Comments:

*1. Raw data. My major problem with the paper is the lack of presentation of the 'raw' station data, figure's 6 and 8 are a synthesis of the entire dataset which whilst interesting should not be how the reader sees the data. There is a 4 page table of  $\delta^{18}O$  measurements and 3 pages of Mg/Ca which is a lot of work that the authors have done, that shouldn't just be in table format in the supplement! I would like to see the data plotted per station (I think in the main text, but also could be in the supplement though it might get overlooked) so that the reader can see how the various stations/species isotope values 'evolve'. For instance, a figure (5x8 panel) of the 5 multinet stations depth T,  $\delta^{18}O$ , and salinity profiles with the values of each species in a different panel. Or the authors could extend figure 2 to include the isotopes/trace metal geochemical values. I understand that these plots also include filter and sediment values which may explain the rationale of the authors for plotting the data like it is.*

Answer: We thank the referee for this comment. However, we think the presentation of the raw data for each station is too much within the main text, which is the reason why we plot the data for each species together. Anyway, we agree that some information is "lost" and therefore we provide some detailed plots in the new supplement S6.

*2. Mg/Ca- $d^{18}O$ . Does it really work? The authors show that they have a  $r$  value of 0.78 and 0.77 for  $d^{18}O_{sw}$  and salinity observed vs expected and the results in Figure 9 gives compelling evidence for the use of Mg/Ca- $d^{18}O$  to estimate  $d^{18}O_{sw}$ . In figure 9a the range in  $d^{18}O$  estimates is more than 0.5 per mil but its difficult to tell whether this could be due to the spread in the station data. As per my previous comment I believe you need to show the individual station data, as it's impossible to link the salinity,  $d^{18}O_{sw}$  and estimates from individual stations to one another. The authors should check the significance of the  $r$  values, for  $n = 6$  (degrees of freedom are  $n-2$ ) the significance value of  $r$  at an alpha levels of 0.5 and 0.1 is 0.812 and 0.917 respectively. It would also help to propagate the errors between the two analytical measurements. Furthermore, Figure 9b, the scale of the y and x axis differ considerably, the entire data's x-axis range is approximately one tick along the y-axis. It would also appear that were the authors to draw a 1:1 line it would be offset from the line the authors have drawn through the data. Combining these two points it appears there is a weak relationship that isn't statistically significant and the approach doesn't exactly predict the 'real values'. In addition, in Figure 9b it would appear that only one axis is 'reversed', the current view of the plot, at a glance without looking at the absolute values along either axis, gives the impression of a negative correlation instead of the positive correlation that it is and the authors state (Pg. 10 Line 1). Why not plot the Figure 3b line ( $d^{18}O_{sw}$  vs salinity) in Figure 9c? Just from eye-balling it I would say that the slopes are different. Or convert the  $d^{18}O_{sw}$  into salinity (though I will admit that might impose some circular reasoning)? I would say that the authors data and perhaps this approach doesn't provide accurate  $d^{18}O_{sw}$  estimates. Nor do they elaborate upon the influence of test-size upon their estimates, how will this be influenced if foraminiferal size is not static through time (Peeters et al., 1999 Mar Micro; Metcalfe et al., 2015 Biogeosciences) and if this result is dependent upon pooling different size fraction ( $>300 \mu m$ ) measurements.*

Answer: We thank the referee for all the remarks above. However, we think that the Mg/Ca- $\delta^{18}\text{O}$  relationship does work, even though we admit to be more careful in our interpretation. We add two new Figures: 9c and 9d (see below) showing the relationship between estimated  $\delta^{18}\text{O}$  and measured  $\delta^{18}\text{O}$  and salinity for each station. Furthermore, we calculated the p values for the average dataset (n=6; degree of freedom 4), which results in p values <0.08 for both relationships (Fig. 9b). This indicates that we have no statistically significant relationship. One reason might be the small number of data, which don't have a large salinity/ $\delta^{18}\text{O}_{\text{seawater}}$  range. The different scale between the x and y axis does not influence the outcome of the relationship, however we plot a 1:1 scale for Fig. 9c and 9d. Fig. 3b shows the relationship between salinity and  $\delta^{18}\text{O}_{\text{seawater}}$  of the upper 600 m of the water column, which is not the same range as for *G. sacculifer*. On the last point, we agree on the effect of the test-size (statistically significant for  $\delta^{18}\text{O}$  of *G. sacculifer* see Fig. 5). Unfortunately, we do not have enough data for all test-sizes to give further information on this very important topic.



NEW: Figure 9: c) Relationship between  $\delta^{18}\text{O}_{\text{seawater}}$ -estimates (foraminiferal tests) and measured  $\delta^{18}\text{O}_{\text{seawater}}$  (seawater) for each station. d) Relationship between  $\delta^{18}\text{O}_{\text{seawater}}$  -estimates (foraminiferal tests) and measured salinity for each station.

3. Mg/Ca. First the amazing supplementary figures: S2 Figure 1 and Figure 2 would be better in the text than Figure 7, however these figures show that there is a lot of scatter within the data generated that should be elaborated upon in the text. I disagree with the authors that (Pg. 8 Lines 3 – 5): “Our Mg/Ca ratios of eight species collected at specific ocean temperature ranges (corresponding to different water depth intervals) are in good agreement with established species-specific Mg/Ca temperature calibrations (Fig 7 cf. Supplement intervals) and further support the foraminiferal Mg/Ca-dependency on ambient water temperature”. The plot of *G. menardii* in S2 Figure 2 could be best described as ‘shotgun’ like; *G. tumida* is recording 5oC (Figure 8) and *G. ungulata* appears to be getting warmer with depth (Figure 8). The authors themselves state: (Pg. 9 Line 7) “the offset between. . .vary from -3C to 9C”; (Pg. 9 Line 28) “the Mg/Ca temperature of fossil tests (~19C) represent the calculated average habitat temperature (~21.7C) far better than the living foraminifers”. But this is not a bad thing, this is the data and the authors should present it a bit differently (e.g. Pg 10 Line 19 “datasets agree well to published d18O and Mg/Ca calibrations”, do they?if these are species specific calibrations should they have offsets?). I think the authors should add an x-axis error bar in S2 Figure 2, but also consider that collected foraminifer may not

have actually calcified in the collected interval so perhaps extend this error bar to incorporate the temperature of shallower depths. Such an approach might explain some of the high Mg/Ca values in the lower temperatures as specimens that had yet to calcify in the water they were caught in. Could this explain the discrepancy with recorded temperatures? One assumption the authors have made is that living foraminifera caught in a net interval are calcifying in it, for filter and the shallowest net it can be assumed that the bulk of the shell (considering that some foraminifera could ascend to the surface during their juvenile stage) comes from that net interval. This assumption doesn't hold true for the deeper nets. It would be interesting to consider the difference between living and dead shell geo-chemistry (in this or another paper), and whether by choosing only living foraminifera the results could be biased (just because it is alive doesn't mean it has to represent the values it was caught in). Highlighting the shell concentration for each net interval could indicate whether the deeper depths are shells of living foraminifera sinking out- side of their habitat zone but still alive (see Peeters et al., 2002; *Global and Planetary Change – for examples e.g. Figures 5 and 6*). This is alluded to by the dashed red-line in Figure 8, which shows the weighted average living depths shallower than some of the measured depth intervals for some species.

Answer: S2 Figure 1 and 2 do not show exactly our interpretation in the main text. We do not believe that all foraminifers precipitate their tests in the sampling depth where we collected the individuals (see *G. sacculifer* P8 L26–27; P6L33–38). This is exactly the reason why we have higher Mg/Ca temperatures estimates for some specimens. For example: Mg/Ca values for *G. menardii* fit pretty well the Mg/Ca estimates from the sediment and the calculated average habitat depth, however, we have high Mg/Ca values of specimens deeper in the water column (>250 m water depth; Fig. 8). Therefore, we can assume that *G. menardii* does not calcify deeper in the water column than 250 m water depth, even though we found a specimen alive (we add this assumption in our manuscript P9L30). The species *G. unguolata* has an increase of Mg/Ca of 0.02 mmol/mol (bulk samples see Table 3), which is smaller than the analytic precision. We point out that specimens of *G. tumida*, which are collected in the deeper water column, have a variable crust (see P9L41), which lowers Mg/Ca values. Furthermore, most of our living foraminifers did not yet finish their life cycle, which results in different Mg/Ca values. Counting all these aspects together, we believe our data agree well with existing calibrations and give reasonable Mg/Ca values.

We added an x-axis error bar in S2 Figure 2.

### Minor Comments

Check the plural and singular of the word foraminifera throughout the text, as well as V-PDB and V-SMOW (sometimes it's PDB)

Done

Pg. 1 Line 8 (first line of Abstract): The first line (slightly) contradicts the second line (if it is successfully approximated, why is refinement needed?) perhaps change 'are successfully' to 'can be' and add 'with varying success' to the end of the sentence

Done

Pg. 1 Line 15 add 'with respect' between disequilibria and to

Done

Pg. 1 Line 36: Avoid starting a sentence with Mg/Ca change to "The ratio of Mg/Ca"

Done

Pg. 2 Line 5: add 'for proxy users' after critical

Done

Pg. 2 Line 7: Perhaps a re-wording? Whilst, relatively few (isotope) geochemical stud- ies have been conducted on recent/living planktic foraminifers, either collected from the water column or culture under

controlled conditions, these studies are important for assessing different controlling factors on  $\delta^{18}\text{O}$  calcite and Mg/Ca during biomineralization.

Done

Pg. 2 Line 7: Also 'relatively few'? I would disagree with few  $\delta^{18}\text{O}$  studies, though Mg/Ca maybe.

Done

Pg. 2 Line 12: 'Here we' instead of 'We here'

Done

Pg. 2 Line 21 move 'and fossil' to the end of the line (so it reads 'below the ship, and fossil foraminifera from sediments')

Done

Pg. 2 line 32 – 34: Why poison  $\delta^{18}\text{O}$  samples? Does the addition of poison impact cavity-ringing down / infrared spectroscopy, as there is some suggestion in the literature that salinity may have an impact on the vaporiser unit (especially changing salinity between samples but also certain salt solutions)

Answer: The water samples were poisoned during the cruise due to the fact that further parameters will be measured on the same samples (e.g.  $\delta^{13}\text{C}_{\text{DIC}}$ ). We agree, it is not necessary for  $\delta^{18}\text{O}$  measurements.

Pg. 2 line 29/Pg. 3 line 1: Bradshaw (1957) did this massive plankton net study/database of the Pacific, halfway through though he stopped using Rose Bengal to identify 'living' as cytoplasm in the shell was just as efficient. Out of curiosity, is there a reason why (Pg. 3 Line 1) 'cytoplasm-bearing' was picked rather than stained? Is there any potential analytical error associated with staining?

Answer: We had stained and unstained samples. However, to identify living planktic foraminifers it is not necessary to stain the sample with Rose Bengal. The cytoplasm itself is very well visible, which indicates that the foraminifer was still alive during sampling (often greenish-yellowish colour). There is no potential error between stained and unstained samples, however, staining impedes the recognition of reddish tests (e.g. *G. ruber* pink, *G. rubescens*; see Jentzen et al., 2018).

Pg. 2 line 40: perhaps add 'trilobus-like' to better describe forms of *T. sacculifer* with a 'spherical last chamber'.

We added this information.

Pg. 2 line 41: *Globorotalia unguolata*, the arch that distinguishes it from *G. menardii* did you perchance consider laser ablating the arch and the rest of the chamber separately? More out of curiosity, it looks like a chamber feature rather than an embellishment (like a keel) so I wonder is it identical to the rest of the chamber but thicker? Or does it have some structural modification that can be observed in the ablation profile.

Answer: We thank the referee for this very interesting comment. Unfortunately, we did not analyse the arch/rim of *G. unguolata* itself.

Pg. 2 line 41: *Globorotalia truncatulinoides dextral*, have you seen the new paper by Reynolds et al (2018; Mar. Micro.)? Did you distinguish between crusted and encrusted *G. truncatulinoides*? Is there any ablation data that might shed light on the Mg/Ca content of encrusted and non-encrusted from your data?

Answer: We did distinguish between crusted and encrusted specimens. However, we did not measure encrusted *G. truncatulinoides* from the plankton tows, but we measured encrusted specimens of *G. tumida*, which had very low Mg/Ca values (see P9 L40).

Pg. 3 Lines 1-4. Add in size fraction used for analysis (from the table it appears not all size fractions picked on pg. 2 line 37 were included in the  $\delta^{18}\text{O}$  analysis).

We added this information.

*Pg. 3 Line 14. The magazine/turret of a Kiel device is quite small, perhaps give an indicator of the number of standards per run or in total measured? (as per the Mg/Ca analysis i.e. Pg. 4 Line 22)*

We added this information.

*Pg. 3 Line 18. Add Picarro before model (I assume it's a Picarro instrument based upon L1102-i).*

Yes, we added this information.

*Pg. 3 Line 26. Whose rearrangement of Kim and O'Neil?*

We added this information.

*Pg. 3 Line 31. Add in a mean number of specimens with a plus/minus.*

Done

*Pg. 4 Line 2. The steps were repeated several (1-2) times to completely remove the cytoplasm, could varying this step have any impact on the resultant values between samples that have had a single step and a replicate step?*

Answer: We cannot see any impact on our data by repeating the oxidation step. However, if the samples would not be clean enough, we would expect too high Mg/Ca values caused by the large amount of cytoplasm/organic material from plankton samples.

*Pg. 4 Line 21. Outliers are classified as those values that fall 2 stdev above or below the mean, but that would mean based upon the proportion without (assuming normality) that ~4.55% of the data could be removed. What's the rationale for this? How much does this influence the data?*

Answer: It does not influence the mean values of our laser ablation measurements. However, this method removes the outliers for the LA-ICP-MS-profiles.

*Pg. 6 Line 18. The transition from juvenile to neanic to adult stages occurs between 100 and 200 um (Brummer et al., 1986 Mar Micro; 1987 Nature), therefore foraminifera above >200 um (species dependent) should be considered as 'adult'. Could the vital effect, described here, instead be due to growth rate (adult specimens with different sized chambers could suggest variation in growth).*

Answer: We thank the referee for this comment. We agree specimens with a test size >200  $\mu\text{m}$  should be noted as adult specimens. However, we think that ontogeny (different stages of growth) influences the isotopic fractionation, which is caused by different metabolism, but also by different numbers of symbionts (e.g. we can expect higher numbers of active symbionts for larger specimens).

*Pg. 7 Line 1. Regarding GAM, if you assume that a shell is a weighted average, if 25% of the shell is composed of gam-calcite and it has a 0.5 per mil offset from living, if you consider it proportionally the gam-calcite would need to have a significantly larger offset from the rest of the living shell.*

Answer: Unfortunately, we cannot give any information of the  $\delta^{18}\text{O}$  values of the GAM calcite, based on our dataset (unknown  $\delta^{18}\text{O}$ -GAM and unknown % of GAM/individual). GAM calcite of 25% is an estimation for *Orbulina universa*, however, not all individuals/species in the surface sediment have GAM calcite, or up to 25%.

*Pg. 7 Line 34. Would it not be appropriate to (like the palaeotemperature d18O eq1) compare the expected Mg/Ca value by calculating an expected Mg/Ca value (assuming that Mg and Ca concentrations are not limiting and using the values in S2 Table 2)*

Answer: We thank the referee for this comment. However, we think it makes more sense to show the estimated Mg/Ca-temperature (which is species-specific). The ambient seawater temperature is the main environmental factor, which influences the Mg/Ca in foraminiferal tests, and not the concentration of Mg or Ca in the water (if we assume the concentrations are not limited in our case). Based on that we can note

the offset between the estimated Mg/Ca temperature and the seawater temperature (as we can see the offset between  $\delta^{18}\text{O}_{\text{calcite}}$  and  $\delta^{18}\text{O}_{\text{equilibrium}}$ , which is T-dependent).

*Figure 1. It's a beautiful figure. However, two things, (1.) the map has a transparency that the scale bar does not, could make it difficult to interpret the color, (2.) The rainbow is a bit difficult for colour blind readers to see (I am guilty of this as well). See: <https://www.climate-lab-book.ac.uk/2014/end-of-the-rainbow/>*

Answer: We thank the referee for this important comment. 1. We changed the scale bar to transparent. 2. We are aware of the problem for colour-blind readers. We produced the map with ODV program, which gives the rainbow colour automatically, and we regret that we cannot change it to other colours.

*Figure 3. Why not plot the figure like Figure 2 (with isotope, salinity and equilibrium plotted per station) rather than as a composite plot?*

Answer: We plot all stations together because we use the data combined in Fig. 6/8/9



# Mg/Ca and $\delta^{18}\text{O}$ in living planktic foraminifers from the Caribbean, Gulf of Mexico and Florida Straits

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**Abstract.** Past ocean temperatures and salinities **can be** approximated from combined stable oxygen isotopes ( $\delta^{18}\text{O}$ ) and Mg/Ca measurements in fossil foraminiferal tests with **varying success**. To further refine this approach, we collected living planktic foraminifers by net sampling and pumping of seasurface waters from the Caribbean Sea, the eastern Gulf of Mexico, and Florida Straits. Analyses of  $\delta^{18}\text{O}$  and Mg/Ca in eight living planktic species (*Globigerinoides sacculifer*, *Orbulina universa*, *Neogloboquadrina dutertrei*, *Pulleniatina obliquiloculata*, *Globorotalia menardii*, *Globorotalia unguolata*, *Globorotalia truncatulinoides* and *Globorotalia tumida*) were compared to measured in situ properties of the ambient seawater (temperature, salinity and  $\delta^{18}\text{O}_{\text{seawater}}$ ) and fossil tests of underlying surface sediments. “Vital effects” such as symbiont activity and test growth cause  $\delta^{18}\text{O}$  disequilibria **with respect** to the ambient seawater and a large scatter in foraminiferal Mg/Ca. Overall, ocean temperature is the most prominent environmental influence on  $\delta^{18}\text{O}_{\text{calcite}}$  and Mg/Ca. Enrichment of the heavier  $^{18}\text{O}$  isotope in living specimens below the mixed layer and in fossil tests are clearly related to lowered in situ temperatures and gametogenic calcification. Mg/Ca-based temperature estimates of *G. sacculifer* indicate seasonal maximum accumulation rates on the seafloor in early spring (March) at Caribbean stations and later in the year (May) in the Florida Straits, related to the respective mixed layer temperatures of  $\sim 26^\circ\text{C}$ . Notably, *G. sacculifer* reveals a **weak** positive linear relationship between foraminiferal derived  $\delta^{18}\text{O}_{\text{seawater}}$  estimates and both measured in situ  $\delta^{18}\text{O}_{\text{seawater}}$  and salinity. Our results affirm the applicability of existing  $\delta^{18}\text{O}$  and Mg/Ca calibrations for the reconstruction of past ocean temperatures and  $\delta^{18}\text{O}_{\text{seawater}}$  reflecting salinity due to the convincing accordance of proxy data in both living and fossil foraminifers, and in situ environmental parameters. Large “vital effects” and seasonally varying proxy signals, however, need to be taken into account.

## 1 Introduction

Calcite tests of planktic foraminifers are precipitated from the surrounding seawater and their stable oxygen isotope compositions ( $\delta^{18}\text{O}_{\text{calcite}}$ ) and Mg/Ca ratios are established proxies to reconstruct past ocean conditions (e.g. Erez and Luz, 1983; Nürnberg et al., 2000). The  $\delta^{18}\text{O}_{\text{calcite}}$  signature depends on the ambient seawater temperatures and oxygen isotopic compositions ( $\delta^{18}\text{O}_{\text{seawater}}$ ) the planktic organism is thriving in. Their relationship was defined in several  $\delta^{18}\text{O}$ -paleotemperature equations (e.g. Erez and Luz, 1983; Bouvier-Soumagnac and Duplessy, 1985; Bemis et al., 1998). Earlier studies showed that  $\delta^{18}\text{O}_{\text{calcite}}$  reveals an offset to the equilibrium of the seawater, caused by environmental factors (e.g. salinity, carbonate ion concentration  $[\text{CO}_3^{2-}]$ , ocean pH) and/or biological controlled processes, so-called “vital-effects” (Weiner and Dove, 2003) (e.g. symbionts photosynthesis, respiration) as influencing factors (Spero and Lea, 1993; Spero et al., 1997; Bemis et al., 1998; Bijma et al., 1999). **Symbiont activity, for example, causes a depletion of  $\delta^{18}\text{O}_{\text{calcite}}$  (e.g. Spero and Lea, 1993). Different ontogenetic stages of the foraminifers result in variable stable isotopes ( $\delta^{18}\text{O}_{\text{calcite}}$  and  $\delta^{13}\text{C}_{\text{calcite}}$ ), whereas higher  $\delta^{18}\text{O}_{\text{calcite}}$  and  $\delta^{13}\text{C}_{\text{calcite}}$  values are measured in tests of adult specimens (e.g. Spero and Lea, 1996).**

The ratios of Mg/Ca in foraminiferal tests are predominantly controlled by ocean temperature. Meanwhile, robust planktic species-specific calibrations exist (e.g. Nürnberg, 1995; Nürnberg et al., 1996; Lea et al., 1999; Anand et al., 2003; Regenberg et al., 2009), which allow to reconstruct the thermal structure of the entire water column, even on timescales of millions of years. The incorporation of magnesium during calcification is largely driven by physiological processes, which may cause Mg/Ca heterogeneity in single tests with high and low Mg-bands in some species (Erez, 2003; Sadekov et al., 2005; Bentov and Erez, 2006; Hathorne et al., 2009; Spero et al., 2015). Further, environmental parameters (e.g. salinity, [CO<sub>3</sub><sup>2-</sup>], ocean pH) may affect foraminiferal Mg/Ca (Nürnberg et al., 1996; Lea et al., 1999; Russel et al., 2004; Kisakürek et al., 2008). Most critical for proxy users are carbonate dissolution processes that considerably lower Mg/Ca in foraminiferal tests (Brown and Elderfield, 1996; Rosenthal et al., 2000; Regenberg et al., 2006). Other influencing aspects, such as variable calcification depths (e.g. vertical migration through the water column during the life cycle of individuals; see Lohmann and Schweitzer, 1990; Schiebel and Hemleben, 2017) or variable seasonal abundances play a major role for the interpretation of stable isotope and Mg/Ca signals of planktic foraminifers (e.g. Tedesco et al., 2007).

Whilst, relatively few geochemical studies (e.g. Mg/Ca) have been conducted on recent/living planktic foraminifers, either collected from the water column or cultured under controlled laboratory conditions, these studies are important for assessing different controlling factors on  $\delta^{18}\text{O}_{\text{calcite}}$  and Mg/Ca during biomineralization (e.g. Kahn, 1979; Erez and Honjo, 1981; Nürnberg et al., 1996; Lea et al., 1999; Russel et al., 2004; Kisakürek et al., 2008; Spero et al., 2015).

Here we systematically sampled the upper water column of the Caribbean, the eastern Gulf of Mexico, and Florida Straits for living tropical and subtropical planktic foraminifers using plankton nets and on board pumping devices.  $\delta^{18}\text{O}_{\text{calcite}}$  and Mg/Ca analyses within bulk calcite and single chambers of living specimens collected from different depth intervals were i) related to ocean parameters (temperature, salinity,  $\delta^{18}\text{O}_{\text{seawater}}$ ) measured in water samples from CTD sampling stations nearby, and ii) compared to fossil counterparts from underlying or nearby surface sediments. Our integrated approach aims to evaluate (i) “vital-effects” under natural conditions, (ii) the ontogenetic development in particular test growth and (iii) the impact of environmental conditions on foraminiferal  $\delta^{18}\text{O}_{\text{calcite}}$  and Mg/Ca to further substantiate their potential as paleoceanographic proxies.

## 2 Material and Methods

### 2.1 Sampling and preparation of planktic foraminifers

Analyses were performed on living foraminifers sampled from plankton nets, pumping from below the ship, and fossil foraminifers from surface sediments obtained during cruises SO164 (RV *Sonne*) in May/June 2002 (Nürnberg et al., 2003) and M78/1 (RV *Meteor*) in February/March 2009 (Schönfeld et al., 2011) (Fig. 1; Table 1). To collect living planktic foraminifers, the Hydrobios Midi multiple opening-closing plankton net (MSN) with a mesh size of 100  $\mu\text{m}$  was deployed at five stations in different water depth intervals (surface to max. 400 m) (Table 1). Further sampling of living specimens was accomplished by pumping seawater from 3.5 m water depth during ship’s transit and subsequent filtering over a 63  $\mu\text{m}$  sieve (PF samples). Immediately after sampling, the plankton samples (MSN and PF) were preserved in a mix of 50 % ethanol and seawater. The MSN samples were stained with Rose Bengal (2 g/l). Surface sediment samples were recovered by Multicorer and USNEL giant box corer at positions close to the MSN stations (Table 1). During cruise M78/1, salinity and temperature were recorded by the RBR XR-420 Conductivity-Temperature-Depth (CTD) profiler and by the shipboard thermosalinograph (Fig. 2). For stable isotope analyses in seawater ( $\delta^{18}\text{O}_{\text{seawater}}$ ), water samples were collected at different water depths (Table 1) with the shipboard rosette Niskin bottle system connected to the CTD profiler, filled in glass bottles (100 ml) and poisoned with 0.2 ml HgCl<sub>2</sub> to prevent biological activity.

In the laboratory (GEOMAR, Kiel), the plankton net samples were rinsed with tap water and all foraminifers were picked wet with a glass pipette. The picked foraminifers were dried on a filter paper at room temperature, fractionated into different



mesh sizes (100–125, 125–150, 150–250, 250–300, 300–400, 400–500 and >500 µm) and identified on species level after Bé (1967) and Schiebel and Hemleben (2017). For isotope and geochemical analyses, individual tests from eight different species were selected including: *Globigerinoides sacculifer* (i.e., *Trilobatus sacculifer*; Spezzaferri et al., 2015) with a spherical last chamber (*G. trilobus* morphotype), *Orbulina universa*, *Neogloboquadrina dutertrei*, *Pulleniatina obliquiloculata*, *Globorotalia menardii*, *Globorotalia unguolata*, *Globorotalia truncatulinoides* dextral, and *Globorotalia tumida* (Supplement S5). Only test size fractions >250 µm and cytoplasm-bearing specimens with an intact calcite test were considered for analyses, indicating that the foraminifers were still alive when collected. For all species, the weighted average living depth (m) and habitat (=living) temperature (°C) (temperature at the weighted average living depth) was calculated based on standing stocks (individual m<sup>-3</sup>) in the water column (Table 2; cf. Jentzen et al., 2018).

Surface sediment samples were freeze-dried, wet sieved using tap water over a 63 µm sieve, and dried at 40 °C. Single intact tests were picked from the 355–400 µm size fraction, to be directly comparable with published data from similar Caribbean station sites (existing δ<sup>18</sup>O<sub>calcite</sub> data from Steph et al., 2009 and Mg/Ca data from Regenberg et al., 2006).

## 2.2 Stable isotope analyses

Depending on the selected species and size fraction, a varying number of specimens were analysed for stable isotopes (δ<sup>18</sup>O<sub>calcite</sub> and δ<sup>13</sup>C<sub>calcite</sub>) (cf. Supplement S1). Prior to the measurements, the foraminiferal tests were cracked and the remaining cytoplasm was removed with a needle. The measurements were run on a ThermoScientific MAT 253 mass spectrometer connected to an automatic carbonate preparation device Kiel CARBO IV at GEOMAR. The stable isotope results are reported relative to the Vienna Pee Dee Belemnite (V-PDB) in per mil (‰) and calibrated versus the National Bureau of Standards (NBS) 19. The in house standard (Solnhofen limestone) run multiple times and after every ten measurements with every magazine of samples and gives a long-term analytic precision of <0.06 ‰ (±1σ) for δ<sup>18</sup>O<sub>calcite</sub> and <0.03 ‰ (±1σ) for δ<sup>13</sup>C<sub>calcite</sub>, respectively.

Stable oxygen isotopes in seawater (δ<sup>18</sup>O<sub>seawater</sub>) were analysed by the Isotope Ratio Infrared Spectroscopy (IRIS) analyser (Picarro Model L1102-i CRDS) at the laboratory of GeoZentrum Nordbayern (Erlangen) (Van Geldern and Barth, 2012). The measurements are expressed in per mil (‰) versus the Vienna Standard Mean Ocean Water (VSMOW). The analytical precision is better than 0.05 ‰ (±1σ).

The difference between the predicted inorganic calcite δ<sup>18</sup>O signal of the seawater (calcite formed in thermodynamic equilibrium, δ<sup>18</sup>O<sub>equilibrium</sub>) and the δ<sup>18</sup>O<sub>calcite</sub> value of the foraminifer is commonly termed the “vital effect” (δ<sup>18</sup>O<sub>disequilibrium</sub>) (Table 2):

$$\delta^{18}\text{O}_{\text{disequilibrium}} = \delta^{18}\text{O}_{\text{calcite}} - \delta^{18}\text{O}_{\text{equilibrium}} \quad (1)$$

To determine δ<sup>18</sup>O<sub>equilibrium</sub> (Fig. 3a), the temperature equation of Kim and O’Neil (1997) for inorganic precipitation was applied follow the relationship after Wilke et al. (2006):

$$\delta^{18}\text{O}_{\text{equilibrium}} = 25.778 - 3.333 * (43.704 + T)^{0.5} + \delta^{18}\text{O}_{\text{seawater}} \quad (2)$$

with in situ temperatures (°C) measured during cruise M78/1 by CTD and measured seawater (δ<sup>18</sup>O<sub>seawater</sub>) values (Schönfeld et al., 2011; Supplement S1). δ<sup>18</sup>O<sub>seawater</sub> was corrected to the V-PDB scale by subtracting 0.27 ‰ after Hut (1987).

## 2.3 Mg/Ca analyses

The ratios of Mg/Ca in foraminiferal calcite were analysed from both bulk samples comprising numerous of tests (on average +/-25 specimens) of a single species, and single specimens, depending on their abundances (cf. Supplement S1). Prior to analyses, the samples were cleaned with a hydrogen peroxide-cleaning step following Barker et al. (2003), which is suggested to be an efficient method to remove the high amount of cytoplasm in live foraminifers (Pak et al., 2004). We

omitted a reductive hydrazine cleaning step as this step is unnecessary for plankton samples. Furthermore, employing only the oxidative cleaning step allows for direct comparison to foraminiferal Mg/Ca from surface sediments, which are treated similarly (Regenberg et al., 2006). For each bulk sample (plankton net and sediment), ~400–800 µg of *G. sacculifer*, *N. dutertrei* and *G. unguolata* from different size fractions were used for analyses (Supplement S1). The tests were gently  
5 crushed between two glass plates, in order to open the chambers, and transferred into a vial. The samples were first rinsed with ultrapure water and ethanol, including an ultrasonic treatment. Then, 250 µl of a NaOH/H<sub>2</sub>O<sub>2</sub> solution (100 µl 30 % H<sub>2</sub>O<sub>2</sub> and 10 ml NaOH) were added to each vial and placed for 20 minutes in a hot water bath (92 °C). For the plankton samples these steps were repeated 1–2 times in order to completely remove the cytoplasm. The samples were subsequently rinsed with ultrapure water. Finally, the tests were leached with 250 µl of HNO<sub>3</sub> (0.001 M). Prior to the element analyses,  
10 the samples were dissolved in HNO<sub>3</sub> (0.075 M). The measurements were performed with an axial-viewing VARIAN 720 Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) at GEOMAR. The data of the measurements were normalised and trend-corrected using the ECRM 752-1 standard (3.761 mmol mol<sup>-1</sup> Mg/Ca; Greaves et al., 2008). The analytic precision is 0.1 mmol mol<sup>-1</sup> (±2σ).

Single chambers of live collected foraminifers were analysed with an Excimer ArF 193 nm laser ablation system, coupled to  
15 an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS Agilent 7500cx) at GEOMAR. Single foraminifers were cleaned with a buffered hydrogen peroxide solution, in a similar way as the bulk samples. Only one specimen was put into a vial to avoid breaking the test during the cleaning process. Each test was rinsed with ultrapure water and ethanol before adding 250 µl of NaOH/H<sub>2</sub>O<sub>2</sub> solution. The samples were then placed in a hot water bath (92 °C) for 20 minutes and rinsed with ultrapure water and ethanol afterwards. Subsequently, the samples were dried at room temperature. The laser ablation  
20 technique allowed us to ablate through the test wall from the outer test surface towards the inner side. Its spot size diameter was focused to 50 and 75 µm. Ablation profiles were carried out on the last four chambers (F to F-3) (Supplement S1). The energy density of the laser was 0.9–2.6 J cm<sup>-2</sup> and a laser repetition rate of 5 and 7 Hz was selected. The following isotopes were measured: <sup>24</sup>Mg, <sup>26</sup>Mg, <sup>27</sup>Al, <sup>43</sup>Ca, <sup>44</sup>Ca, <sup>55</sup>Mn, <sup>66</sup>Zn, <sup>88</sup>Sr, <sup>232</sup>Th and <sup>238</sup>U. The ablation was stopped when the test wall was penetrated. Analyses were calibrated using standard glasses 610 and 612 of National Institute of Standards and  
25 Technology (NIST) using the values of Jochum et al. (2011). The NIST 610 and NIST 612 were ablated with an energy density of 2–3 J cm<sup>-2</sup> after every ten measurements of foraminiferal tests. Raw counts of elements were processed offline and <sup>43</sup>Ca was used as internal standard to account for ablation yield. Outliers (average value ±2σ) were rejected from the results. A powder pellet of JCT-1 (giant clam shell) was used as reference and repeatedly analysed (n=15) during the ablation sessions revealing an average Mg/Ca ratio of 1.21 ± 0.13 mmol mol<sup>-1</sup> (standard deviation of 10.6 %, 1σ) being consistent  
30 with the consensus of solution analyses in many laboratories (Mg/Ca=1.289 mmol mol<sup>-1</sup> Hathorne et al., 2013).

In situ temperatures (°C) measured during cruise M78/1 (Schönfeld et al., 2011) were compared to derived Mg/Ca-temperature estimates. We applied different calibrations for each species to account for species-specific differences (e.g. Russel et al., 2004; Cléroux et al., 2008; Regenberg et al., 2009; cf. Supplement S2).

#### 2.4 Calculation of δ<sup>18</sup>O<sub>seawater</sub>

35 The combination of δ<sup>18</sup>O<sub>calcite</sub> and Mg/Ca in foraminiferal tests allows us to estimate δ<sup>18</sup>O of the ambient seawater (Craig and Gordon, 1965; Schmidt, 1999; Fig. 3b), which is used as a proxy for surface seawater salinity. We compared our measured in situ δ<sup>18</sup>O<sub>seawater</sub> to δ<sup>18</sup>O<sub>seawater</sub> estimates derived from combined foraminiferal δ<sup>18</sup>O<sub>calcite</sub> and Mg/Ca-temperatures of *G. sacculifer*. For the calculation we used the species-specific δ<sup>18</sup>O-paleotemperature equation for *G. sacculifer* of Spero et al. (2003) with the species-specific Mg/Ca-temperature calibration for *G. sacculifer* of Regenberg et al. (2009).

## 2.5 Calcite dissolution

Calcite dissolution can affect foraminiferal Mg/Ca and stable isotopes ( $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ ) as a function of the regionally different calcite saturation states in the oceans and the sensitivity of the species-specific test structure (Brown and Elderfield, 1996; Spero et al., 1997; Zeebe, 1999; Bijma et al., 1999; Regenberg et al., 2006; 2014). The calcite saturation state  $\Delta[\text{CO}_3^{2-}]$  is defined as:

$$\Delta[\text{CO}_3^{2-}] = [\text{CO}_3^{2-}]_{in-situ} - [\text{CO}_3^{2-}]_{saturation} \quad (3)$$

and decreases from the surface ( $\sim 150\text{--}200 \mu\text{mol kg}^{-1}$ ) to  $\sim 5000$  m water depth ( $< 0 \mu\text{mol kg}^{-1}$ ) in the eastern Caribbean Sea and Gulf of Mexico (Fig. 4).  $\Delta[\text{CO}_3^{2-}]$  of  $\sim 21 \mu\text{mol kg}^{-1}$ , which is a critical threshold for the onset of selective  $\text{Mg}^{2+}$  ion removal from planktic foraminiferal calcite, is at  $\sim 2500\text{--}3000$  m water depth in the study area. Below this, the undersaturated waters generally lower foraminiferal Mg/Ca through preferential dissolution (Regenberg et al., 2006; 2014). Furthermore, increasing  $[\text{CO}_3^{2-}]$  concentrations and seawater pH cause decreasing  $\delta^{18}\text{O}_{\text{calcite}}$  and  $\delta^{13}\text{C}_{\text{calcite}}$  values of the foraminiferal tests (Spero et al., 1997; Zeebe, 1999; Bijma et al., 1999). As all plankton net samples of this study were taken from shallower than 400 m water depth, the studied living foraminifers originate from supersaturated seawater with respect to calcite ( $\Delta[\text{CO}_3^{2-}] > 50 \mu\text{mol kg}^{-1}$ ) and that substantial  $\text{Mg}^{2+}$  ion removal (loss of higher Mg/Ca calcite) is not to be expected. This is not valid for fossil tests from surface sediments below 2500–3000 m water depth. At these locations (Stations M78/1-220/SO164-22-2 and M78/1-219/SO164-02-3) we use the dissolution corrected Mg/Ca values from Regenberg et al. (2006; 2009) (cf. Supplement 1).

## 3 Results and Discussion

### 3.1 Hydrographical setting during sampling

In order to be able to directly relate our results on vertical foraminiferal distribution patterns and species-specific (isotope) geochemical signatures to the modern hydrographic conditions in the study area, we also took temperature, salinity and  $\delta^{18}\text{O}_{\text{seawater}}$  measurements. The CTD and thermosalinograph data gathered during cruise M78/1 (February–March 2009) reveal low sea surface temperatures (SST) in the Gulf of Mexico ( $\sim 20^\circ\text{C}$ ) and Florida Straits ( $\sim 24^\circ\text{C}$ ) (Fig. 1; 2) comparable to the boreal winter situation (Fig. 2; Locarnini et al., 2013). Hydrographic conditions in the Caribbean vary seasonally with a large range of SSTs (range in the Florida Straits up to  $5^\circ\text{C}$ ) and salinities (SSS; range in the Caribbean Sea up to 1 (psu)) (Fig. 2) and are closely linked to the migrating Intertropical Convergence Zone (ITCZ), which is at its northernmost position ( $6\text{--}10^\circ\text{N}$ ) during summer (Locarnini et al., 2013; Zweng et al., 2013). The surface mixed layer extends to max. 100 m water depth in the Caribbean and is characterised by the relatively fresh Caribbean Water (CW;  $< 36$  psu). The lowest salinity is recorded in the southeastern Caribbean during summer and autumn when the Amazon and Orinoco river discharge is most intense and freshwater plumes arrive in the Caribbean Sea (Wüst, 1964; Müller-Karger et al., 1989; Chérubin and Richardson, 2007). Modified CW is transported via anticyclonic eddies (Loop Current) towards the Gulf of Mexico and Florida Straits (Vukovich, 2007). In the upper thermocline, the highly saline Subtropical Under Water (SUW;  $> 37$  (psu)) prevails. This water mass originates in tropical and subtropical regions (Gallegos, 1996; Blanke et al., 2002) and resides in  $\sim 80\text{--}160$  m water depth. The  $18^\circ\text{C}$  Sargasso Sea Water (Eighteen Degree Water = EDW) prevails in  $\sim 200\text{--}400$  m water depth entering the Caribbean Sea via the passages of the Greater Antilles (Morrison and Nowlin, 1982). The Gulf Common Water ( $\sim 23^\circ\text{C}$  and  $\sim 36.4$  (psu); Vidal et al., 1994) possibly influences the Florida Straits hydrography (Station 210/211) in the upper thermocline at  $100\text{--}150$  m, characterised by low salinity (36.5 (psu)).

Seawater  $\delta^{18}\text{O}$  ( $\delta^{18}\text{O}_{\text{seawater}}$ ) averages to  $\sim 0.9\text{‰}$  (VSMOW) in the uppermost 400 m water depth (Fig. 3a). Highest  $\delta^{18}\text{O}_{\text{seawater}}$  values (1.3 ‰) can be found in the salinity maximum at  $\sim 60\text{--}150$  m water depth, whereas the lowest value (0.3 ‰) is measured in the deepest sample at the lowest salinity. Additionally, the in situ  $\delta^{18}\text{O}_{\text{seawater}}$  and salinity recorded during M78/1

show a positive correlation (linear regression,  $r = 0.81$ ) and yield similar values as earlier data sets from the Caribbean Sea (Schmidt et al., 1999) (Fig. 3b). The  $\delta^{18}\text{O}_{\text{equilibrium}}$  increases with depth from  $\sim -1.5$  to  $1$  ‰ in dependence of the decreasing ocean temperature (Fig. 2; 3a).

### 3.2 Vital effects on foraminiferal $\delta^{18}\text{O}_{\text{calcite}}$

- 5 In order to address the effects of symbiont activity and life cycle on the foraminiferal oxygen isotopes,  $\delta^{18}\text{O}_{\text{calcite}}$  values of living foraminifers were compared to the calculated  $\delta^{18}\text{O}_{\text{equilibrium}}$  of the ambient seawater and  $\delta^{18}\text{O}_{\text{calcite}}$  estimates of fossil tests from underlying surface sediments.

#### 3.2.1 Symbionts and life cycle effect on foraminiferal $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$

Specimens of *G. sacculifer* and *O. universa* from the mixed layer are characterised by large negative  $\delta^{18}\text{O}_{\text{disequilibrium}}$  values of  
10  $-0.35$  ‰ and  $-0.32$  ‰, respectively (Table 2). These two species host dinoflagellates as symbionts (Gastrich, 1987) and similarly negative  $\delta^{18}\text{O}_{\text{disequilibrium}}$  values were reported in spinose, symbiont-bearing species caught in plankton tows from various ocean areas (Table 2 and references therein). Laboratory experiments (Spero, 1992; Spero and Lea, 1993; Bemis et al., 1998) revealed a depletion of  $0.3$  to  $0.6$  ‰ in  $\delta^{18}\text{O}_{\text{calcite}}$  of *O. universa* and *G. sacculifer* under high irradiance levels related to algae photosymbiont activity. In particular, a high irradiance in the euphotic zone intensifies the photosynthetic  
15 rate in the Caribbean Sea under its prevailing oligotrophic conditions (Spero and Parker, 1985; Morel et al., 2010). Enhanced photosymbiont activity increases the  $\text{O}_2$  concentration and fosters  $\text{CO}_2$  fixation, resulting in an elevated pH within the microenvironment around the living foraminifer (Jørgensen et al., 1985; Rink et al., 1998). Both, increasing pH and increasing carbonate ion concentration  $[\text{CO}_3^{2-}]$  apparently cause a depletion of  $\delta^{18}\text{O}_{\text{calcite}}$  (Spero et al., 1997; Bijma et al., 1999).

20 Among all species studied, only *G. sacculifer* and *N. dutertrei* reveal a significant positive correlation (Spearman rank correlation,  $p < 0.05$ ) between test size and stable isotopes ( $\delta^{18}\text{O}_{\text{calcite}}$  and  $\delta^{13}\text{C}_{\text{calcite}}$ ) (Fig. 5, Supplement S3), suggesting that ontogeny affects the isotopic fractionation processes. The species *G. unguolata* shows lower  $\delta^{18}\text{O}_{\text{calcite}}$  values in the test size fraction  $< 300$   $\mu\text{m}$  and *G. menardii* indicate no significant ontogenetic effect ( $p > 0.5$ ; Fig. 5). It should be noted that for some species we did not have enough sample material in all test size classes. However, our results are consistent to Kahn (1979),  
25 Kahn and Williams (1981), Spero and Lea (1996) and Bemis et al. (1998), who postulated that juvenile foraminifers have a larger “vital-effect” than adult individuals, with their tests being depleted of the heavy  $^{18}\text{O}$  and  $^{13}\text{C}$  isotopes due to a higher metabolic rate (incorporation of respired  $\text{CO}_2$ ) and/or rapid growth rate. Rapidly growing calcitic skeletons result in a stronger kinetic isotope fractionation and cause the depletion of heavier  $^{18}\text{O}$  and  $^{13}\text{C}$  isotopes (McConnaughey, 1989).

Vertical migration of planktic species to deeper and colder water masses during their life cycle may additionally affect  
30  $\delta^{18}\text{O}_{\text{calcite}}$ , leading to commonly higher values in adult specimens (Kroon and Darling, 1995; Lončarić et al., 2006; Birch et al., 2013). Samples from the same test size fraction of all species exhibit the enrichment of heavier  $^{18}\text{O}$  isotopes at deeper water levels (Fig. 6; Table 3). We speculate that the increasing  $\delta^{18}\text{O}_{\text{calcite}}$  at deeper water levels is a function of increasing  $\delta^{18}\text{O}_{\text{equilibrium}}$  of the ambient seawater, rather than ontogenetic effects itself. The surface dweller *G. sacculifer* reveals the largest  $\delta^{18}\text{O}_{\text{disequilibrium}}$  value ( $\sim 1$  ‰) in the thermocline (Table 2). As a higher rate of photosynthetic processes in deeper  
35 water depths can be excluded and specimens were still alive when sampled, we suggest that *G. sacculifer* completed calcifying in the thermocline before reproduction. Our observation corroborates South Atlantic plankton net studies of Lončarić et al. (2006), who noted that *G. sacculifer*  $\delta^{18}\text{O}_{\text{calcite}}$  increased with depth in the upper 60 m water depth and remained constant below the surface mixed layer, even though  $\delta^{18}\text{O}_{\text{equilibrium}}$  increased continuously.

### 3.2.2 The $\delta^{18}\text{O}$ offset between living and fossil foraminifers

It becomes evident that almost all fossil tests from surface sediment samples, in particular *N. dutertrei*, *P. obliquiloculata*, *G. truncatulinoides* and *G. tumida* are enriched in  $\delta^{18}\text{O}_{\text{calcite}}$  ( $>0.5\text{‰}$ ) compared to their living counterparts from the water column (Fig. 6; Table 3).  $\delta^{18}\text{O}_{\text{calcite}}$  of fossil shallow dwellers *G. sacculifer* and *O. universa* are rather close to those values of specimens caught in the thermocline (average difference of  $0.14\text{‰}$  and  $0.02\text{‰}$ , respectively) (Table 3). Yet, the overall discrepancy in  $\delta^{18}\text{O}_{\text{calcite}}$  between fossil and living specimens may be best explained by gametogenetic calcification processes or calcite crust formation, which take place during the vertical migration through the water column. At the end of the life cycle and prior to gametogenesis, various planktic foraminifer species (including *G. sacculifer*, *O. universa*, *P. obliquiloculata*, *G. truncatulinoides*, *G. tumida*) add a calcite crust of variable thickness on the outer surface of the test (Schiebel and Hemleben, 2017, and references therein). Based on calculations of Bouvier-Soumagnac and Duplessy (1985) and Hamilton et al. (2008) up to 25 % ( $\sim 4\text{ }\mu\text{g}$ ) gametogenic calcite is added by *O. universa*, which is mainly secreted in colder waters prior to reproduction. The tests thereby lose their glassy and transparent appearances (Bé, 1980; Deuser et al., 1981; Duplessy et al., 1981b; Hemleben et al., 1985; Schweitzer and Lohmann, 1991). Specifically, spinose species resorb their spines before releasing their gametes (Bé and Anderson, 1976; Spero, 1988). These processes result in heavier  $\delta^{18}\text{O}_{\text{calcite}}$  compositions of fossil tests from surface sediments (and even individual foraminifers from sediment traps) (Duplessy et al., 1981b; Bouvier-Soumagnac and Duplessy, 1985; Bouvier-Soumagnac et al., 1986; Lin et al., 2011). Consistently, the heavy  $\delta^{18}\text{O}_{\text{calcite}}$  values in adult specimens of *G. truncatulinoides* and *G. tumida* may be best explained by vertical migration into colder water masses at a late ontogenetic stage (Franco-Fraguas et al., 2011; Birch et al., 2013). Orr (1967) and Vergnaud-Grazzini (1976) recognised that living individuals of *G. truncatulinoides* with a thick test and pustules on the test surface are more likely to be found in deeper water masses than non-ornamented, thin-shelled specimens. As expected, such tests had  $\delta^{18}\text{O}_{\text{calcite}}$  values close to those observed in surface sediments. Overall, our proxy database supports the notion that specimens of *P. obliquiloculata*, *G. tumida* and *G. truncatulinoides* add a thick opaque calcite layer or cortex at deeper water depths than  $\sim 400\text{ m}$ . Hence, the fossil tests are enriched in  $\delta^{18}\text{O}_{\text{calcite}}$  relative to the living foraminifers (up to  $0.85\text{‰}$ ) (Fig. 6; Table 3).

During the sampling campaign in February/March 2009, mainly juvenile specimens of *N. dutertrei* were found in plankton nets (mode test size fraction  $150\text{--}250\text{ }\mu\text{m}$ ; Jentzen et al., 2018). This finding may additionally explain the large  $\delta^{18}\text{O}_{\text{calcite}}$  offset between living foraminifers and fossil tests ( $\sim 1\text{‰}$ ) (Fig. 6; Table 3). Kroon and Darling (1995) recognised that small specimens of *N. dutertrei* have similar  $\delta^{18}\text{O}_{\text{calcite}}$  values as surface dwellers and lower values than large specimens, supporting the notion on the ontogenetic related migration to deeper waters. Fairbanks et al. (1982) and Bouvier-Soumagnac and Duplessy (1985) also noted increasing  $\delta^{18}\text{O}_{\text{calcite}}$  values of *N. dutertrei* with increasing water depth in the Panama Basin and Indian Ocean, suggesting that this species secrete substantial proportions of their tests below the mixed layer. Furthermore, living *N. dutertrei* from the South China Sea were depleted in  $\delta^{18}\text{O}_{\text{calcite}}$  compared to individuals from sediment traps (Lin et al., 2011). Our data confirm these assumptions as we recognised higher  $\delta^{18}\text{O}_{\text{calcite}}$  values and larger individuals of *N. dutertrei* in surface sediments compared to the mixed layer (Fig. 6; Table 3; Jentzen et al., 2018).

The species *G. menardii* show increasing  $\delta^{18}\text{O}_{\text{calcite}}$  values from the mixed layer to the thermocline ( $+0.3\text{‰}$ ), and from the thermocline to the surface sediments ( $+0.2\text{‰}$ ) pointing to decreasing ambient seawater temperatures at deeper water levels and migration within the water column (Fig. 2; Table 3). Apparently, *G. unguolata* is an exception to the rule, as this species does not show the enrichment of  $\delta^{18}\text{O}_{\text{calcite}}$  in fossil tests compared to living specimens (Fig. 6; Table 3). Yet, the species secreted their calcite tests close to the equilibrium with the ambient seawater ( $0.01\text{--}0.08\text{‰}$ ) throughout the water column (Table 2). The average surface sediment  $\delta^{18}\text{O}_{\text{calcite}}$  value corresponds well with the depth where the highest standing stock was observed during the sampling campaign in February/March 2009 (Fig. 6; Jentzen et al., 2018).

### 3.3 Mg/Ca-based ocean temperature assessment from living foraminifers

In order to evaluate Mg/Ca as proxy for seawater temperature, we compared Mg/Ca-temperature estimates of living specimens to (i) measured in situ temperatures and (ii) Mg/Ca-temperature estimates of fossil tests from surface sediments. Within this study, Mg/Ca analyses were performed on bulk foraminiferal samples measured by ICP-OES and single tests measured by LA-ICP-MS. ICP-OES samples of *G. sacculifer*, *N. dutertrei* and *G. unguolata* yield higher Mg/Ca ratios on average compared to LA-ICP-MS samples from the same MSN sample (Table 3). The data indicate a difference of  $0.5 \pm 0.5$  mmol mol<sup>-1</sup> for *G. sacculifer* (average value of eight MSN sampling intervals), 1.2 mmol mol<sup>-1</sup> for *N. dutertrei* (one MSN sampling interval) and  $0.17 \pm 0.05$  mmol mol<sup>-1</sup> for *G. unguolata* (three MSN sampling intervals). We compare the results of both methods to each other having in mind the data discrepancy originating from the different analytical techniques. For LA-ICP-MS only small amounts of foraminiferal calcite from single chambers are analysed and for the ICP-MS the bulk calcite from whole foraminiferal tests are measured.

Our Mg/Ca ratios of eight species collected at specific ocean temperature ranges (corresponding to different water depth intervals) are in good agreement with established species-specific Mg/Ca-temperature calibrations (Fig. 7; cf. Supplement S2), and further support the foraminiferal Mg/Ca-dependency on ambient water temperature. Hence, we estimate Mg/Ca-temperatures applying the best fitting calibration for each species (Fig. 8). Overall, all specimens collected in the surface waters of the eastern Gulf of Mexico (PF samples) yield low Mg/Ca-temperature estimates (averaged  $\sim 20.6$  °C) according to the low early spring temperatures of  $\sim 20$  °C prevailing during cruise M78/1 (Fig. 1). Higher Mg/Ca-temperature estimates ( $\sim 25$  °C) of shallow dwellers (symbiont and facultative symbiont bearing species) in the Florida Straits and Caribbean Sea (MSN samples) point to higher temperatures in the mixed layer ( $> 24$  °C). Low Mg/Ca ratios of deep dwellers (*G. truncatulinoides* and *G. tumida*) in the thermocline follow the decreasing ambient seawater temperatures (Fig. 8).

#### 3.3.1 (Facultative) symbiont bearing species

Our dataset is most complete for *G. sacculifer*, allowing for a detailed comparison between Mg/Ca-based temperature estimates from plankton net and surface sediment samples. In the Caribbean Sea, the estimated Mg/Ca-temperatures for *G. sacculifer* ( $\sim 26$  °C) are consistent with in situ temperatures of the mixed layer ( $\sim 26.2$  °C), the average habitat temperature ( $\sim 26$  °C, derived from the standing stock, Table 2) and Mg/Ca-temperatures derived from fossil tests ( $\sim 26$  °C) (Fig. 8). Below 150 m water depth, the deviation between Mg/Ca-temperature and the ambient seawater temperature increases, which support the former conclusion based on  $\delta^{18}\text{O}_{\text{calcite}}$  that *G. sacculifer* completed calcifying above or within the thermocline. Lower temperature estimates of  $\sim 24$  °C in the Florida Straits (Station 211) (Fig. 7) mirror the generally lower sea surface temperatures of  $\sim 24.6$  °C at this station during cruise M78/1 (Fig. 2). Here the fossil tests from surface sediments yield higher Mg/Ca ratios ( $+0.7$  mmol mol<sup>-1</sup>) than the living specimens. The Mg/Ca-temperature of fossil specimens indicate  $\sim 26.5$  °C, which is rather comparable to temperatures in the Florida Straits of the mixed layer in May (Locarnini et al., 2013, Fig. 2). Foraminiferal census data from the MSN samples suppose that the highest population density of *G. sacculifer*, consequently also the highest flux and accumulation rate of empty tests on the seafloor, appears during early spring in the Caribbean Sea, linking this species to the warm and oligotrophic Caribbean Water (CW) ( $\sim 26$  °C) (Jentzen et al., 2018). Furthermore, high frequencies of *G. sacculifer* are related with the strength of the Loop Current transporting warm Caribbean Water into the Gulf of Mexico (Poore et al., 2013). Therefore, we presume that a higher flux of *G. sacculifer* in Florida Straits is likely to occur later in the year, presumably in May, hence after our sampling, and the fossil tests of *G. sacculifer* from the Caribbean Sea and Florida Straits thereby reflect different seasonal signals.

Beside the seasonal effect, millennial-scale variabilities further affect the Mg/Ca signal of fossil tests from surface sediments. Regenberg et al. (2006) assumed an age range of 2–3 kyrs in surface sediments ( $\sim 0$ –1 cm) of the Caribbean Sea. As such, the surface sediments include the record of earlier climate variations, like the Little Ice Age, when sea surface



temperatures in the Caribbean were cooler by  $\sim 2$  °C (Watanabe et al., 2001). A large scatter of  $\sim 0.9$  mmol mol<sup>-1</sup> Mg/Ca of fossil tests from Caribbean surface sediments was therefore linked partly to past environmental variabilities (Regenberg et al., 2006). Our study, however, shows a similarly large Mg/Ca scatter in living specimens collected from the same plankton nets (MSN samples, Mg/Ca range up to  $\sim 0.87$  mmol mol<sup>-1</sup>; Fig. 7). Furthermore, LA-ICP-MS profiles across single chamber walls reveal a large Mg/Ca variability, with decreasing Mg/Ca values towards the final chamber (F) (cf. Supplement S4), which implies that “vital-effects” drive Mg<sup>2+</sup> incorporation. Earlier studies on surface sediments and culture experiments indicate an ontogenetic effect on the incorporation of Mg<sup>2+</sup> during test growth of *G. sacculifer*, with lowest Mg/Ca ratios in the final, newly precipitated chambers (Sadekov et al., 2005; Dueñas-Bohórquez et al., 2011). Although lower average Mg/Ca ratios ( $\sim 0.3$  mmol mol<sup>-1</sup>) were measured in living specimens than in fossil test, the bulk foraminiferal samples of living *G. sacculifer* from the mixed layer show a significant positive correlation between Mg/Ca and in situ temperatures (Pearson linear,  $r = 0.8$ ,  $p < 0.05$ ), with an overall Mg/Ca scatter comparable to that of fossil specimens from surface sediments (Fig. 7).

Our database for the other species is rather limited. Nonetheless, we can derive the following information. The symbiont bearing species *O. universa* characteristically yields very high Mg/Ca ratios in single tests (up to  $\sim 10$  mmol mol<sup>-1</sup> on average) (cf. Lea et al., 1999; Russel et al., 2004). Mg/Ca-temperature estimates of *O. universa* are on average  $\sim 1$  °C lower than the measured in situ temperature, but show decreasing values in larger depths according to lower in situ temperatures (Fig. 8; Table 3). The offset between Mg/Ca-temperatures of *P. obliquiloculata* and in situ temperatures vary from  $-3$  °C to  $9$  °C. Both, *O. universa* and *P. obliquiloculata* show low and high Mg<sup>2+</sup> bands across single chambers of the tests (Supplement S4). Those bands are likely caused by physiological processes (Eggins et al., 2004; Kunioka et al., 2006; Sadekov et al., 2009; Spero et al., 2015) and reveal a large Mg/Ca variability in single chambers. Single LA-ICP-MS measurements of *N. dutertrei* yield lower Mg/Ca ratios than the ICP-OES measurements (Table 3). Here, the high Mg-heterogeneity in single chambers (cf. Fehrenbacher et al., 2017) probably caused the large offset between the two measuring techniques (see above). However, the average derived Mg/Ca-temperature of plankton bulk samples ( $\sim 26.3$  °C) at station 221 is in good agreement with the in situ temperature of the seawater at this station ( $\sim 26.5$  °C) (Fig. 8). The difference of  $0.71$  mmol mol<sup>-1</sup> Mg/Ca between the living and fossil bulk samples (Table 3) support the notion that adult specimens of *N. dutertrei* dwell at larger depths and continue calcifying (development of a crust; cf. Steinhardt et al., 2015; Fehrenbacher et al., 2017), as indicated by the lower  $\delta^{18}\text{O}_{\text{calcite}}$  values and smaller specimens collected in the upper mixed layer (Jentzen et al., 2018). Living specimens of *G. menardii* yield a Mg/Ca-temperature range between  $\sim 18$  °C and  $26.5$  °C, which is larger but covers the temperature range of fossil tests ( $\sim 23.2$ – $25$  °C) and the calculated average habitat temperature ( $\sim 24.5$  °C; Table 2) in the Florida Straits and Caribbean Sea (Fig. 8). **The high Mg/Ca-temperature estimate in the deep sampling depth interval (Fig. 8) might indicate that *G. menardii* calcify in the upper 250 m water depth.**

### 3.3.2 Symbiont barren species

In the Florida Straits both, bulk and single Mg/Ca measurements of *G. unguolata* yield temperature estimates of  $\sim 24$  °C in the mixed layer and thermocline (Fig. 8) being congruent to the average habitat temperature of  $23.8$  °C during February/March 2009 (Table 2). The average Mg/Ca-temperature estimates of living and fossil *G. truncatulinoides* ( $\sim 19$  °C) mirror the average habitat temperature of  $\sim 20$  °C during February/March 2009 (Fig. 8; Table 2). The deep dweller *G. tumida* shows a decreasing Mg/Ca-temperature trend from the mixed layer to the thermocline following the decreasing in situ temperature (Fig. 8). The fossil tests of *G. tumida* show higher average Mg/Ca ratios than the living individuals (Table 3) and yield higher temperature estimates. However, the Mg/Ca-temperature of fossil tests ( $\sim 19$  °C) represents the calculated average habitat temperature ( $\sim 21.7$  °C) far better than the living foraminifers, which show an offset to the prevailing in situ temperature of  $\sim 7$  °C to  $17$  °C (Fig. 8) most likely due to variable crusting of the chambers (cf. Supplement S4).

### 3.4 $\delta^{18}\text{O}_{\text{seawater}}$ relationship

The combination of foraminiferal  $\delta^{18}\text{O}_{\text{calcite}}$  and Mg/Ca-temperatures to estimate  $\delta^{18}\text{O}_{\text{seawater}}$  approximating paleo-salinity is a commonly accepted approach in paleoceanography (e.g. Lea et al., 2000; Schmidt et al., 2004; Nürnberg et al., 2008). Support derived from living foraminifers collected under natural conditions is still sparse. Our unique dataset on living planktic foraminifers in the mixed layer (>125 m water depth) at least allows us to test the abovementioned approach for the surface dweller *G. sacculifer* from the Caribbean Sea and Florida Straits (Fig. 9). As the  $\delta^{18}\text{O}_{\text{seawater}}$  estimates are strongly depending on both the applied  $\delta^{18}\text{O}$ -paleotemperature equation and empirical Mg/Ca-calibration, we decided to apply the  $\delta^{18}\text{O}$ -paleotemperature equation of Spero et al. (2003). This equation is based on *G. sacculifer* cultured in laboratory, which takes the large disequilibrium of  $\delta^{18}\text{O}_{\text{calcite}}$  in living specimens to the ambient seawater into account (Table 2). For the estimation of Mg/Ca-temperature, we applied the species-specific calibration of Regenberg et al. (2009) for *G. sacculifer* derived from fossil tests of surface sediments in the tropical Atlantic and Caribbean Sea. Our study shows that this calibration reflects our in situ temperatures very close (Fig. 7).  $\delta^{18}\text{O}_{\text{seawater}}$  estimates of *G. sacculifer* show a weak positive linear relationship with in situ  $\delta^{18}\text{O}_{\text{seawater}}$  as well as with salinity for Station 211 and 221 (Fig. 9c; 9d). Station 219 does not show a positive linear relationship between  $\delta^{18}\text{O}_{\text{seawater}}$  estimates of *G. sacculifer* and in situ  $\delta^{18}\text{O}_{\text{seawater}}$  but with salinity (Fig. 9d). The average dataset of all stations shows a weak positive linear relationship (Fig. 9b), however, it is not statistically significant ( $p>0.05$ ;  $p=0.074$  for salinity and  $p=0.069$  for  $\delta^{18}\text{O}_{\text{seawater}}$ , respectively). Additionally, different test-size fractions influence the  $\delta^{18}\text{O}_{\text{seawater}}$  estimates (see 3.2.1 for  $\delta^{18}\text{O}_{\text{calcite}}$ ) and should be taken into account for studies reconstructing past ocean parameters (see Metcalfe et al., 2015). However, our study on living foraminifers hence provides compelling evidence that the combination of foraminiferal  $\delta^{18}\text{O}_{\text{calcite}}$  and Mg/Ca-temperature reflecting ambient seawater properties reliably approximates the modern ocean salinity.

### 4 Conclusions

Our combined stable isotopes ( $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ ) and Mg/Ca analyses on living planktic foraminifers, collected by MSN and PF from surface to max. 400 m water depth of the Caribbean Sea, the eastern Gulf of Mexico and Florida Straits, allow for the following conclusions:

- (1) The large negative disequilibrium (between  $\delta^{18}\text{O}_{\text{calcite}}$  and  $\delta^{18}\text{O}_{\text{equilibrium}}$ ) of up to -0.35 ‰ observed for *G. sacculifer* and *O. universa* point to a strong photosynthetic activity of the host symbionts (dinoflagellates).
- (2) Ontogeny most likely controls  $\delta^{18}\text{O}_{\text{calcite}}$  and  $\delta^{13}\text{C}_{\text{calcite}}$  values. In this study *G. sacculifer* and *N. dutertrei* show a significant increase of  $\delta^{18}\text{O}_{\text{calcite}}$  and  $\delta^{13}\text{C}_{\text{calcite}}$  with increasing test size.
- (3) Vertical migration in the water column and additional secretion of a calcite crust or gametogenic calcite (at the end of the foraminiferal life cycle) likely causes the increase of  $\delta^{18}\text{O}_{\text{calcite}}$  with water depths and the enrichment of heavier  $^{18}\text{O}$  isotopes in fossil tests compared to living specimens.
- (4) The large intraspecific scatter of Mg/Ca implies a strong “vital-effect”. Nonetheless, it is evident that the ambient calcification temperature drives the Mg/Ca compositions in foraminiferal tests and causes lowered Mg/Ca derived temperature estimates at lowered in situ temperature.
- (5) The various species-specific datasets agree well to published  $\delta^{18}\text{O}$  and Mg/Ca calibrations.
- (6) Fossil tests of *G. sacculifer* from surface sediments in the Caribbean Sea and Florida Straits suggest that the regional Mg/Ca signatures may be seasonally biased. Mg/Ca values indicate that the highest flux/accumulation rate of *G. sacculifer* occurs during spring (March) in the Caribbean Sea and delayed by a few months in the Florida Straits (most likely in May) linked to prevailing seawater temperatures of ~26 °C in the mixed layer.

(7) Combined  $\delta^{18}\text{O}_{\text{calcite}}$  and Mg/Ca-temperatures of *G. sacculifer* yield  $\delta^{18}\text{O}_{\text{seawater}}$  estimates, which show a **weak** positive linear relationship with measured in situ  $\delta^{18}\text{O}_{\text{seawater}}$  and salinity.

*Supplement.*

5 S1 Dataset

S2 Calibrations

S3 Statistics

S4 LA-ICP-MS profiles

S5 SEM Plate

10 **S6 Station plots**

*Data availability.* Dataset of this article can be found in the Supplement and in Jentzen et al. (2018), Regenberget al. (2006), and Steph et al. (2009).

15 *Competing interests.* The authors declare that they have no conflict of interest.

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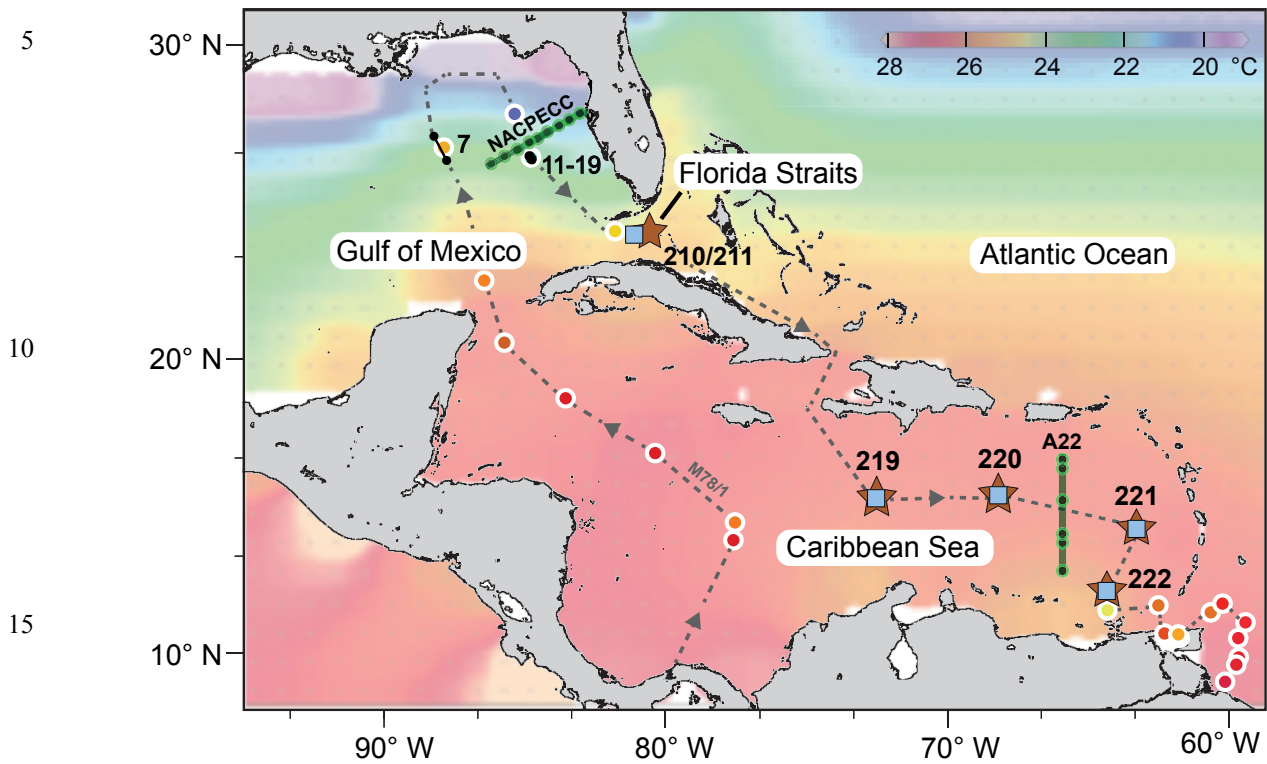


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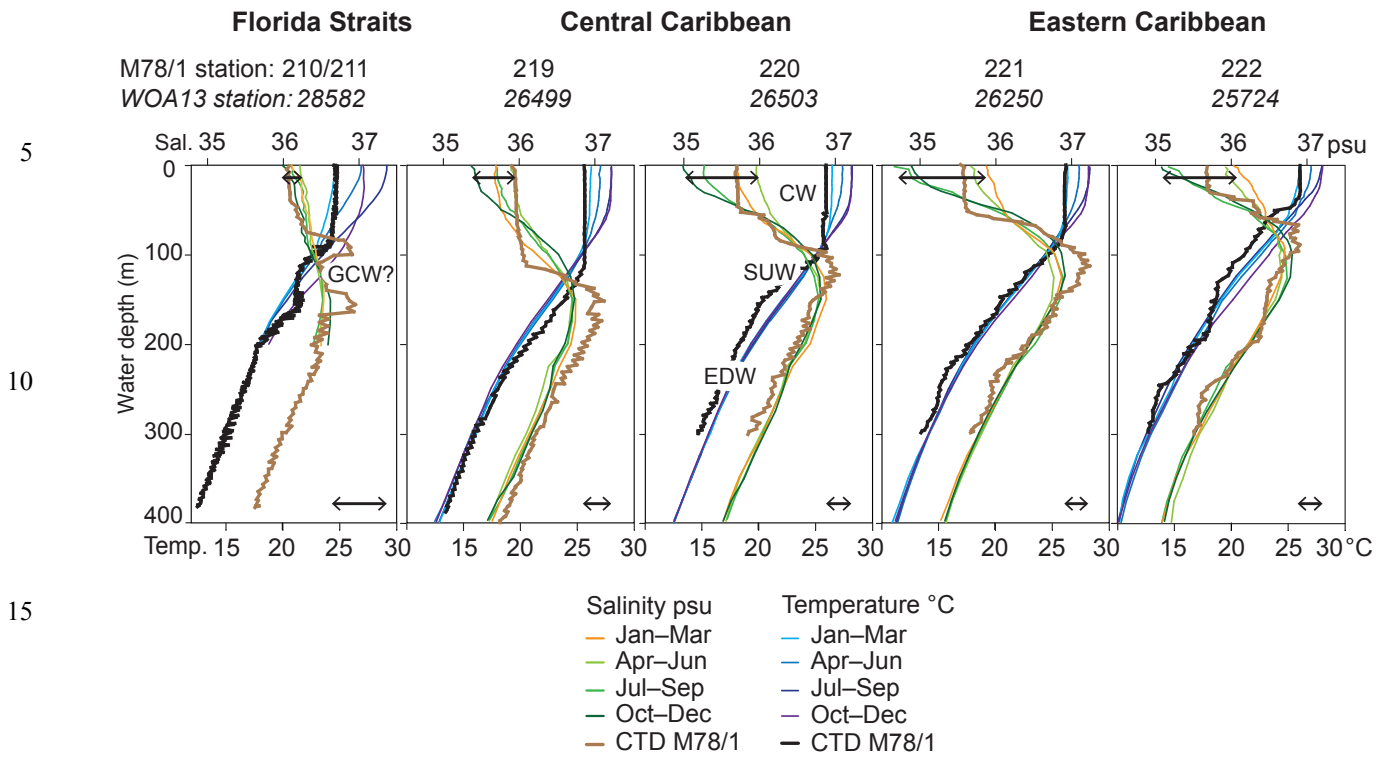
FIGURES



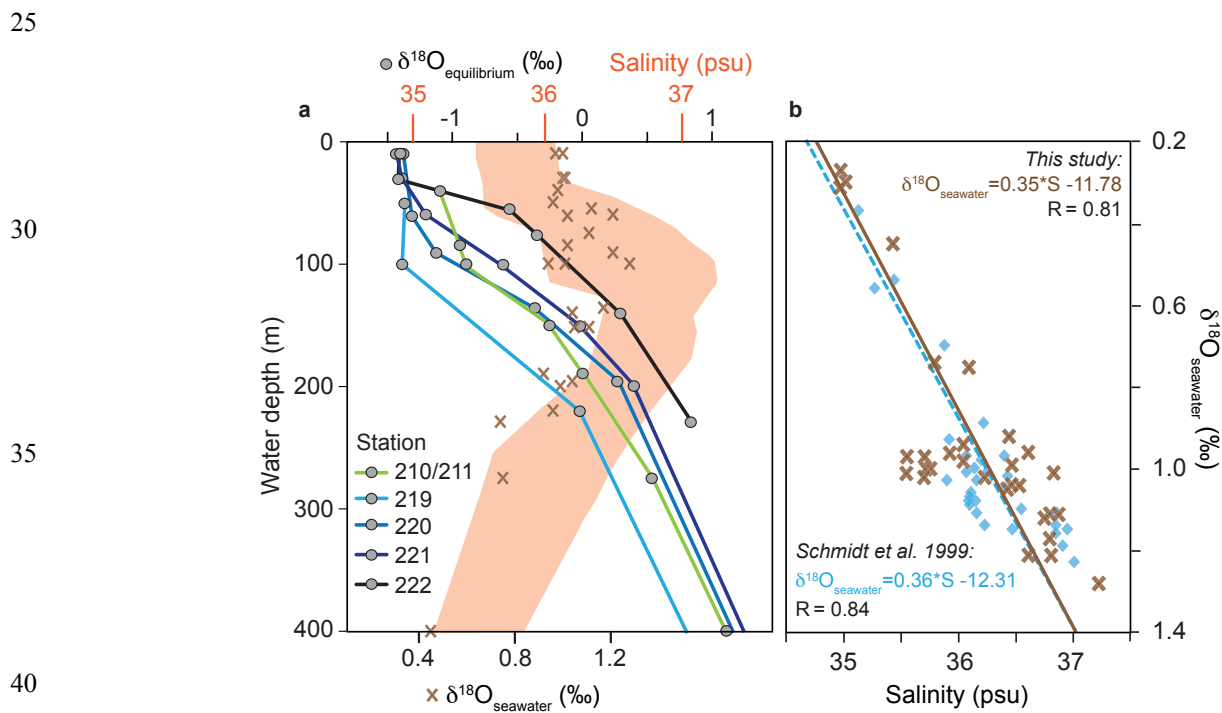
**Figure 1.** Sea surface temperature chart (SST) of the subtropical W-Atlantic (Caribbean Sea, Gulf of Mexico and Florida Straits) showing sampling locations for living planktic foraminifers (Table 1). Brown stars: Multiclosure net samples (MSN) and CTD stations (RV *Meteor* cruise M78/1). Black dots and lines: Plankton filter samples (PF, M78/1). Blue squares: Surface sediment samples (M78/1 and RV *Sonne* cruise SO164, cf. Regenberg et al., 2006; Steph et al., 2009). Green lines and grey dots: World Ocean Circulation Experiment (WOCE) transect line A22 (stations 10–15) and North American Carbon Program (NACP) line NACPECC (stations 20–28) (cchdo.ucsd.edu). Coloured shading: SST illustrated with ODV (Schlitzer, 2009) using World Ocean Atlas 2013 (WOA13) data from January–March (Locarnini et al., 2013). Coloured dots with white outline: SST (3.5 m water depth) recorded during cruise M78/1 with the shipboard thermosalinograph (Schönfeld et al., 2011; Supplement S1). Grey dashed line: Cruise track of RV *Meteor* cruise M78/1 in February and March 2009.

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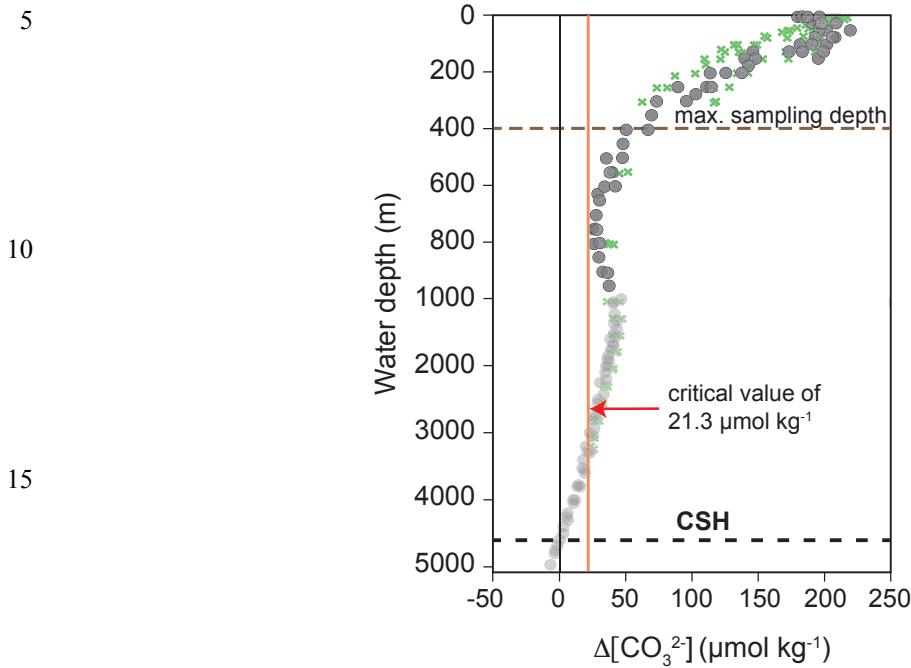


**Figure 2.** Temperature (°C) and salinity (psu) depth profiles in the working area. In situ CTD data measured during cruise M78/1 (March 2009, thick brown and black lines) are presented in comparison to the seasonally differentiated World Ocean Atlas 2013 (WOA13) data (Locarnini et al., 2013; Zweng et al., 2013; coloured thin lines). GCW: Gulf Common Water; CW: Caribbean Water; SUW: Subtropical Under Water; EDW: 18 °C Sargasso Sea Water. Black double arrows indicate the seasonal ranges of temperature (bottom) and salinity (top) in the uppermost water column (0–10 m water depth).



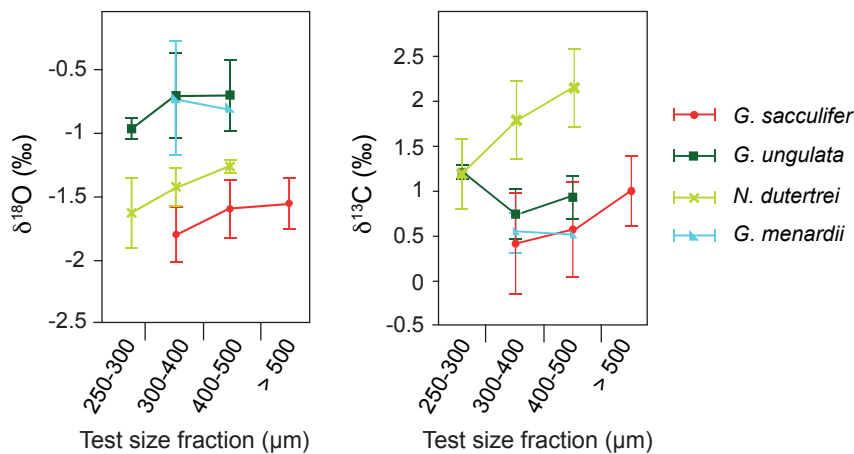
**Figure 3. a)**  $\delta^{18}\text{O}_{\text{seawater}}$  (‰ VSMOW) and colour-coded  $\delta^{18}\text{O}_{\text{equilibrium}}$  (‰ PDB) depth profiles at the CTD stations 210/211, 219, 220, 221, and 222 (see Fig. 1). Red shading: Salinity envelope (psu) of the ambient seawater from Florida Straits and Caribbean Sea measured during cruise M78/1 matching  $\delta^{18}\text{O}_{\text{seawater}}$ . **b)** Brown crosses: Measured in situ salinity vs.  $\delta^{18}\text{O}_{\text{seawater}}$ .

$\delta^{18}\text{O}_{\text{seawater}}$  in the Caribbean Sea and Florida Straits in the upper 600 meter of the water column (cf. Supplement S1 for data); blue squares: Salinity vs.  $\delta^{18}\text{O}_{\text{seawater}}$  from Schmidt et al. (1999; Global Seawater Oxygen-18 Database) in the upper 600 meter of the water column in the Caribbean Sea.



**Figure 4.** Calcite saturation state indicated by  $\Delta[\text{CO}_3^{2-}]$  depth profiles of the Caribbean Sea and Gulf of Mexico. Grey dots and green crosses: Transect A22 (stations 10–15) and NACPECC (stations 20–28) (Fig. 1) with  $\Delta[\text{CO}_3^{2-}]$  being the difference between  $[\text{CO}_3^{2-}]_{\text{in-situ}}$  and  $[\text{CO}_3^{2-}]_{\text{saturation}}$ . Alkalinity and  $\text{TCO}_2$  were taken from WOCE and NACP (cchdo.ucsd.edu; cruise RV *Knorr* in 1997, EXPOCODE: 316N151\_4 and cruise RV *Ronald H. Brown* in 2007, EXPOCODE: 33RO20070710) to calculate  $[\text{CO}_3^{2-}]_{\text{in-situ}}$  using the program CO2SYS (Pierrot et al., 2006; taking the constants ( $K_1$  and  $K_2$ ) of Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and ( $K_{\text{SO}_4}$ ) from Dickson (1990)).  $[\text{CO}_3^{2-}]_{\text{saturation}}$  was calculated after Jansen et al. (2002). Red vertical line indicates the critical  $\Delta[\text{CO}_3^{2-}]$  value of  $21.3 \mu\text{mol kg}^{-1}$  below which selective  $\text{Mg}^{2+}$  ion removal starts (Regenberg et al., 2014); black dashed line marks the calcite saturation horizon (CSH), which is defined to  $0 \mu\text{mol kg}^{-1}$  and represents the top of the lysocline at  $\sim 4600$  m water depth; brown dashed line indicates the maximum plankton tow sampling depth.

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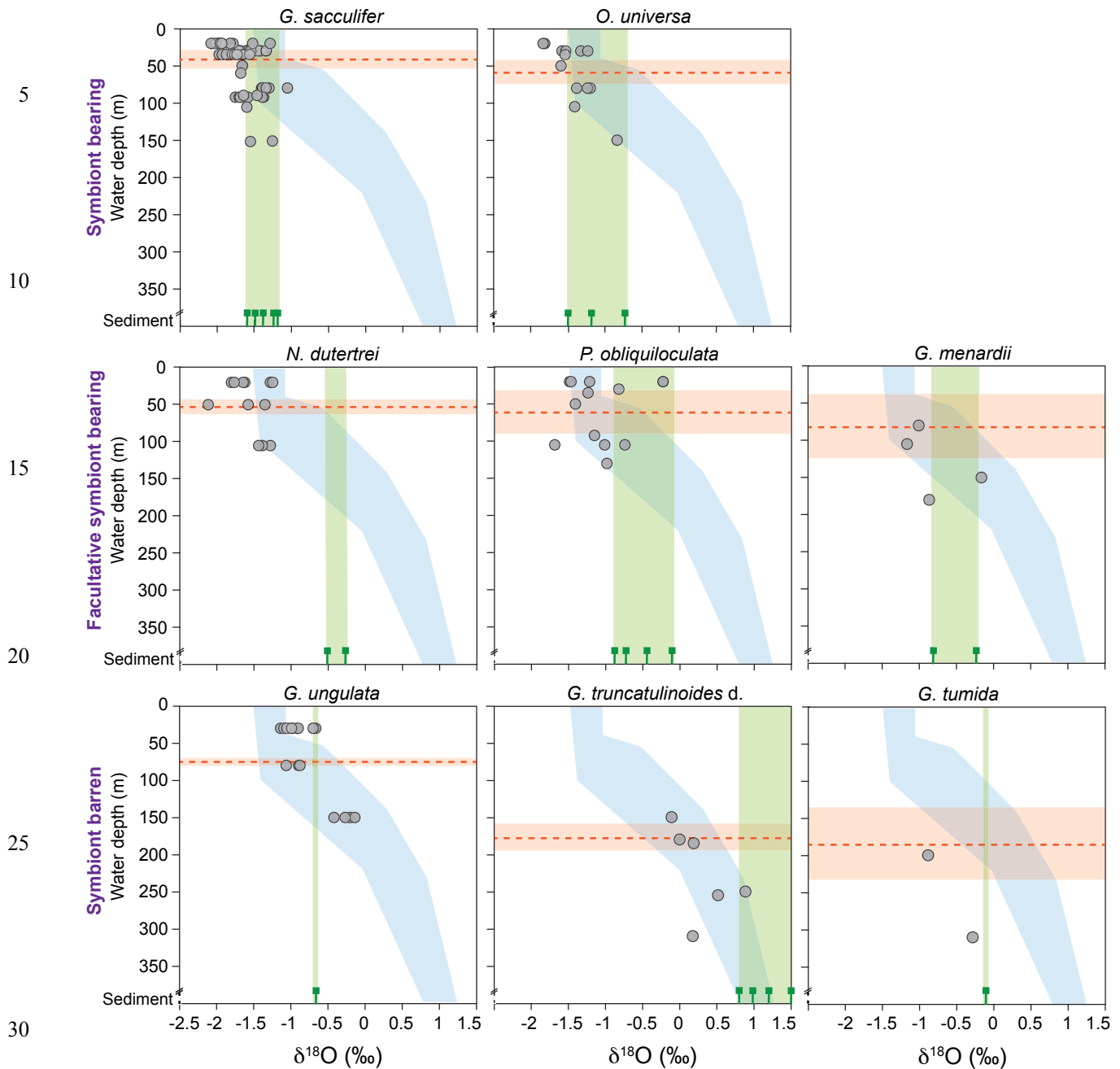


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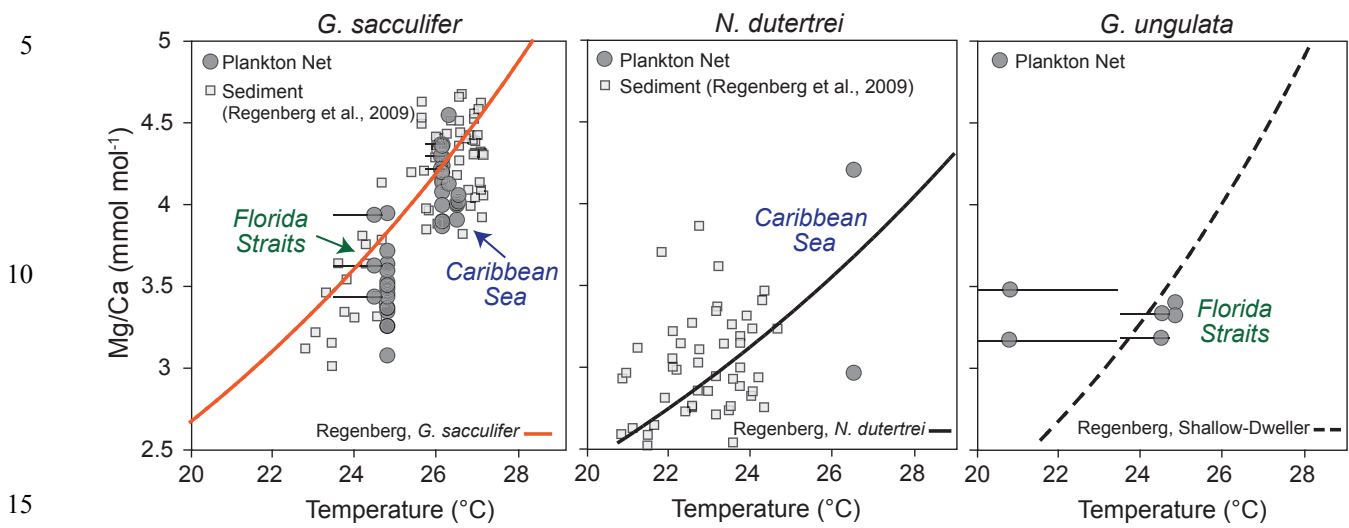
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**Figure 5.** Stable oxygen and carbon isotopes (average  $\delta^{18}\text{O}_{\text{calcite}}$  and  $\delta^{13}\text{C}_{\text{calcite}} \pm$  standard deviations) compared to different test size fractions of living planktic foraminifera (only species with more than one analysed test size fractions are depicted).

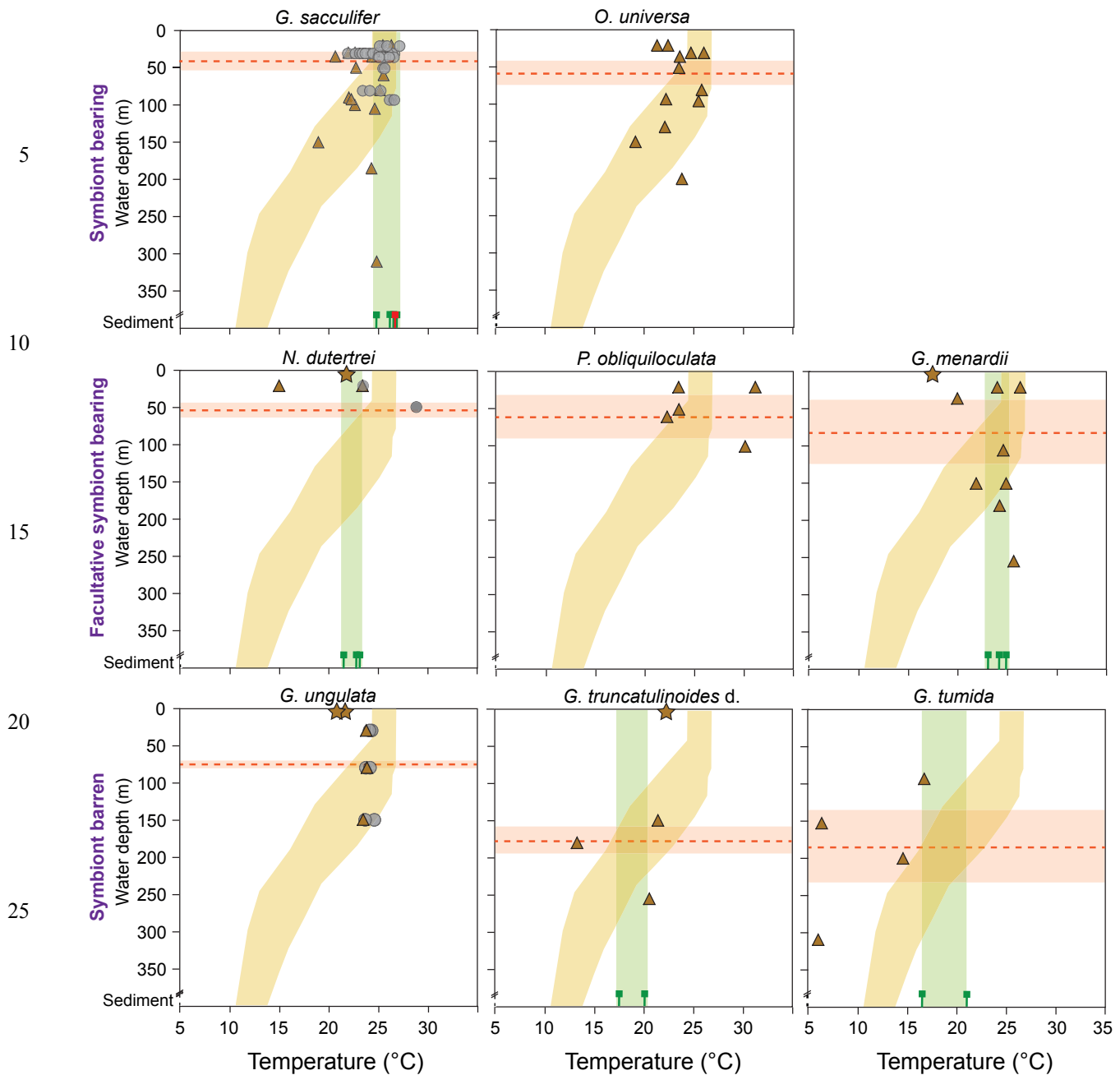




**Figure 6.** Stable oxygen isotopes of living planktic foraminifers from Florida Straits and the Caribbean Sea plotted vs. water depth (m) in comparison to calculated  $\delta^{18}\text{O}_{\text{equilibrium}}$  and surface sediment data (illustrating the “vital effect”). The foraminiferal dataset was differentiated into symbiont bearing, facultative symbiont bearing, and symbiont barren species from top to bottom (Table 2; see Supplement S1 for data). Grey dots: Foraminiferal  $\delta^{18}\text{O}_{\text{calcite}}$  from MSN samples, plotted at the mean sampling depth intervals. Blue shading:  $\delta^{18}\text{O}_{\text{equilibrium}}$  envelope of the ambient seawater from Florida Straits and the Caribbean Sea (cf. Fig. 3a). Green bars: Range of  $\delta^{18}\text{O}_{\text{calcite}}$  of fossil tests from surface sediments (green signs = average values of single stations; cf. Supplement S1). Red dashed lines: Average weighted living depths of single species during the sampling campaign in February/March 2009 (red shaded bars = the standard deviations; Table 2). Note, all test size fractions are included.



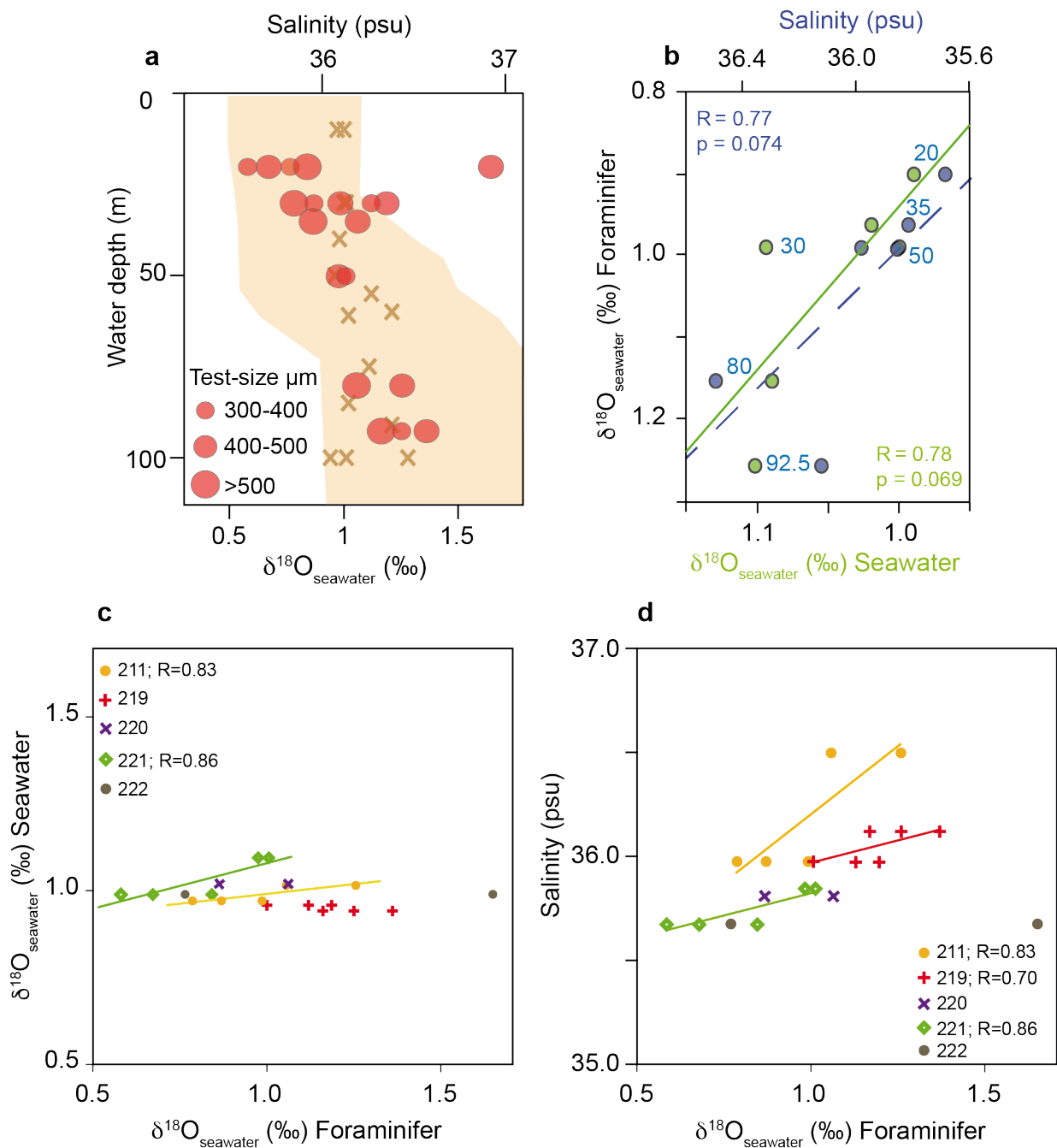
**Figure 7.** Mg/Ca values of ICP-OES bulk samples vs. temperature. Grey dots: Mg/Ca values of living specimens (*G. sacculifer*, *N. dutertrei* and *G. unguolata*), depicted at the average in situ temperature of the plankton net intervals (MSN) in the Florida Straits and Caribbean Sea recorded during cruise M78/1. Black error bars: Modern temperature ranges of the sampling intervals. Grey squares: Mg/Ca ratios of fossil tests vs.  $\delta^{18}\text{O}$  calcification temperature from the Caribbean Sea and tropical Atlantic modified after Regenberg et al. (2009). Orange curve: Mg/Ca calibration of Regenberg et al. (2009) (surface sediments) for *G. sacculifer*. Black curve: Mg/Ca calibration of Regenberg et al. (2009) for *N. dutertrei*. Dashed black curve: Mg/Ca calibration of Regenberg et al. (2009) for shallow dwellers.



30 **Figure 8.** Mg/Ca derived temperature estimates of living planktic foraminifers combined from Florida Straits, the eastern Gulf of Mexico and the Caribbean Sea in comparison to the ambient seawater temperature. The foraminiferal dataset was differentiated into symbiont bearing, facultative symbiont bearing, and symbiont barren species from top to bottom (Table 2; cf. Supplement S1 for data). Grey dots: Mg/Ca-temperature estimates from bulk foraminiferal MSN samples measured by ICP-OES, depicted at the mean sampling depth intervals. Brown triangles and stars: Mg/Ca-temperature estimates derived from LA-ICP-MS measurements of single tests from MSN samples (Caribbean Sea and Florida Straits) and PF samples (Gulf of Mexico), respectively (average values, cf. Supplement S1). Yellow shading: Temperature envelope (°C) of the ambient seawater from Florida Straits and the Caribbean Sea measured during cruise M78/1 (Fig. 2; Schönfeld et al., 2011). Note: PF samples (brown stars) were taken in 3.5 m water depth in the eastern Gulf of Mexico at SST of 20 °C during cruise M78/1 (Fig. 1). Green bars: Mg/Ca derived temperature range of fossil bulk foraminiferal samples from surface sediments closest to the MSN (Green sign: Average values of single stations in the Caribbean Sea; Red sign: average value of *G. sacculifer* in the Florida Straits, cf. Supplement S1). Red dashed lines: Average weighted living depths of single species during the sampling campaign in February/March 2009 (red bars= standard deviation; Table 2). Note, all test size fractions are included.

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5 **Figure 9.**  $\delta^{18}\text{O}_{\text{seawater}}$ -estimates based on foraminiferal tests from living *G. sacculifer* compared to measured  $\delta^{18}\text{O}_{\text{seawater}}$  and  
 10 salinity recorded during cruise M78/1 in the Caribbean Sea and Florida Straits. **a)** Red dots: Average  $\delta^{18}\text{O}_{\text{seawater}}$ -estimates of  
 bulk samples from different test size fractions; brown crosses: in situ  $\delta^{18}\text{O}_{\text{seawater}}$  (‰ VSMOW); orange envelop: Salinity. **b)**  
 Relationship between average  $\delta^{18}\text{O}_{\text{seawater}}$ -estimates (foraminiferal tests), measured  $\delta^{18}\text{O}_{\text{seawater}}$  (seawater) and measured in  
 situ salinity. Blue and green dots indicate average values at a specific water depth (blue numbers denote average sampling  
 water depth in m). **c)** Relationship between  $\delta^{18}\text{O}_{\text{seawater}}$ -estimates (foraminiferal tests) and measured  $\delta^{18}\text{O}_{\text{seawater}}$  (seawater) for  
 each station. **d)** Relationship between  $\delta^{18}\text{O}_{\text{seawater}}$ -estimates (foraminiferal tests) and measured salinity for each station.

**TABLES**

**Table 1.** Station list of sediment, water and plankton samples obtained during cruises SO164 and M78/1 (Nürnberg et al., 2003; Schönfeld et al., 2011). MUC: Multicorer; GKG: Giant box corer; CTD: Conductivity Temperature Depth profiler; MSN: Hydrobios Midi multiple opening-closing plankton net; PF: Plankton filter. \*indicates surface sediment sites close to MSN station (1) 219, (2) 220, (3) 221 and (4) 211 (Fig. 1).

Cruise	Date	Device	Station No.	Latitude N (Start-End)	Longitude W (Start-End)	Water depth (m)	Sampling intervals/depth
SO164	27.05.2002	MUC	02-3 *(1)	15°18.29	72°47.06	2977	0–1 cm
SO164	07.06.2002	MUC	22-2 *(2)	15°24.00	68°12	4506	0–1 cm
SO164	09.06.2002	MUC	24-3 *(3)	14°11.89	63°25.43	1545	0–1 cm
M78/1	10.03.2009	MUC	212-1 *(4)	24°11.10	81°15.74	723	0–1 cm
M78/1	19.03.2009	GKG	222-8	12°1.48	64°28.50	1019	surface
M78/1	10.03.2009	CTD	210-13	24°14.88	80°55.10	452	40, 85, 100, 150, 190, 275, 400 m
M78/1	10.03.2009	CTD	211	24°15.50	80°54.81	456	-
M78/1	15.03.2009	CTD	219-1	15°18.27	72°47.08	2956	50, 100, 220, 600 m
M78/1	16.03.2009	CTD	220-1 220-2	15°23.99 15°23.99	68°12.01 68°11.99	4480 4480	10, 61, 91, 136, 196, 485 m
M78/1	18.03.2009	CTD	221-1 221-2	14°11.89 14°11.98	63°25.45 63°25.41	1534 1534	10, 30, 60, 100, 150, 200, 500 m
M78/1	19.03.2009	CTD	222-1	12°1.49	64°28.55	1023	10, 30, 55, 75, 140, 229 m
M78/1	10.03.2009	MSN	211-5 211-6	24°15.50 24°15.30	80°54.81 80°54.69	456 453	0–60, 60–100, 100–200, 200–300, 300–400 m
M78/1	15.03.2009	MSN	219-7 219-8	15°18.30 15°18.30	72°47.06 72°47.06	2960 2960	0–60, 60–125, 125–180, 180–220, 220–400 m
M78/1	17.03.2009	MSN	220-8 220-9	15°23.99 15°23.99	68°12.00 68°12.00	4481 4482	0–70, 70–110, 110–150, 150–220, 220–300 m
M78/1	18.03.2009	MSN	221-7 221-8	14°11.89 14°11.89	63°25.43 63°25.43	1533 1535	0–40, 40–60, 60–150, 150–210, 210–300 m
M78/1	19.03.2009	MSN	222-6 222-7	12°1.57 12°1.55	64°28.80 64°28.80	1031 1028	0–40, 40–80, 80–120, 120–180, 180–300 m
M78/1	03.03.2009	PF	7	26°31.38–27°39.86	87°5.32–88°16.23	-	3.5 m
M78/1	06.03.2009	PF	11	26°18.35–26°12.21	84°44.97–84°41.92	-	3.5 m
M78/1	06.03.2009	PF	12	26°10.7–26°12.48	84°44.08–84°43.40	-	3.5 m
M78/1	07.03.2009	PF	19	26°12.18–26°12.18	84°43.87–84°43.87	-	3.5 m

**Table 2.** Average weighted living depth (m), habitat temperature (°C), symbionts information and  $\delta^{18}\text{O}_{\text{disequilibrium}}$  values of single species from this study and other authors.

Species	Avg. living depth (m) <sup>x</sup>	Avg. habitat temperature (°C) <sup>x</sup>	Disequilibrium values	
			$\delta^{18}\text{O}_{\text{calcite}}$	$\delta^{18}\text{O}_{\text{equilibrium}}$ (‰)
<i>G. sacculifer</i>	41±9	25.9	-0.36 to -0.03 -0.13 to -0.16 <sup>+</sup>	-0.39
<i>N. duerrei</i>	54±10	25.11	-1.57 to -0.29 <sup>+</sup>	-0.11
<i>O. universa</i>	58±16	25.13	-0.95	
<i>P. obliquicollata</i>	61±29	25.61	-0.24 to 0 <sup>+</sup>	+0.06
<i>G. angulata</i>	75±5	23.81	-0.3	
<i>G. menardi</i>	81±43	24.47	-0.54	
<i>G. truncatulinoides</i>	176±18 <sup>d</sup>	20.14 <sup>d</sup>	-0.15 to +1.28	
<i>G. tumida</i>	185±49	21.72	<0	
Symbionts				
Mixed layer*				
Thermocline*				
Bouvier-Soumagnac and Duplessy, 1985				
Duplessy et al., 1981a				
Erez and Honjo, 1981 <sup>#</sup>				
Vergnaud-Grazzini, 1976				
Kahn, 1979				
Lontaric et al., 2006				
Shackleton et al., 1973				

\* This study (average values); <sup>x</sup> Jenzen et al. (accepted); <sup>d</sup> *G. truncatulinoides* dextral  
<sup>1</sup> = Gástrich, 1987; <sup>2</sup> = Bø, 1977; <sup>3</sup> = Kucera, 2007  
<sup>F</sup> = Facultative symbionts  
<sup>+</sup> = large/thick specimens; <sup>#</sup> = seasonal variations



**Table 3.** Average values of  $\delta^{18}\text{O}_{\text{calcite}}$  and Mg/Ca (measured on ICP-OES\* and LA-ICP-MS) from the mixed layer, thermocline and surface sediment (cf. Supplement S1 for data). PF samples are not included in the calculations.

Species	$\delta^{18}\text{O}_{\text{calcite}}$ (‰)			Mg/Ca (mmol mol <sup>-1</sup> )		
	Mixed layer	Thermocline	Sediment	Mixed layer	Thermocline	Sediment <sup>5</sup>
<i>G. sacculifer</i>	-1.62	-1.52	-1.38	3.87*/ 3.51	3.52	4.20*
<i>P. obliquiloculata</i>	-1.15	-1.07	-0.55	2.84	2.86	
<i>O. universa</i>	-1.53	-1.13	-1.15	8.33	7.61	
<i>N. dutertrei</i>	-1.51	-1.37	-0.4	3.59*/ 2.36		2.88*
<i>G. ungulata</i>	-0.95	-0.26	-0.67	3.30*/ 3.20	3.32*/ 3.10	
<i>G. menardii</i>	-1.01	-0.73	-0.54	3.10	3.19	3.27*
<i>G. tumida</i>		-0.58	-0.11	2.45	1.80	2.68*
<i>G. truncatulinoides</i> d.		0.28	1.13		2.5	2.52*
*Bulk samples (measured on ICP-OES)						