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Salinity control on Na incorporation into calcite tests of the planktonic foraminifera *Trilobatus sacculifer* – Evidence from culture experiments and surface sediments

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Abstract. The quantitative reconstruction of past seawater salinity has yet to be achieved and the search for a direct and independent salinity proxy is ongoing. Recent culture and field studies show a significant positive correlation of Na/Ca with salinity in benthic and planktonic foraminiferal calcite. For accurate paleoceanographic reconstructions, consistent and reliable calibrations are necessary, which are still missing. In order to assess the reliability of foraminiferal Na/Ca as a direct proxy for seawater salinity, this study presents electron microprobe Na/Ca data, measured on cultured specimens of *Trilobatus sacculifer*. The culture experiments were conducted over a wide salinity range of 26 to 45, while temperature was kept constant. To further understand potential controlling factors of Na incorporation, measurements were also performed on foraminifera cultured at various temperatures in the range of 19.5 °C to 29.5 °C under constant salinity conditions. Foraminiferal Na/Ca ratios positively correlate with seawater salinity ($Na/Ca_{foram} = 0.97 + 0.115 \cdot Salinity$, $R = 0.97$, $p < 0.005$). Temperature on the other hand exhibits no statistically significant relationship with Na/Ca ratios indicating salinity to be the dominant factor controlling Na incorporation. The culturing results are corroborated by measurements on *T. sacculifer* from Caribbean and Gulf of Guinea surface sediments. In conclusion, planktonic foraminiferal Na/Ca can be applied as a reliable proxy for reconstructing sea surface salinities, albeit species-specific calibrations might be necessary.

1 Introduction

The combination of foraminiferal Mg/Ca and stable oxygen isotopes ($\delta^{18}O$) measured on the same specimens is a well-established approach to estimate both past sea surface temperatures (SST) and $\delta^{18}O$ of seawater ($\delta^{18}O_{sw}$) (Elderfield and Ganssen, 2000; Anand et al., 2003; Flower et al., 2004). When corrected for past changes in global ice volume and

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individual temperature effects, the ice-volume free $\delta^{18}O_{ivf\text{-}sw}$ indicates past changes in salinity (S), caused by changes in evaporation, precipitation and freshwater fluxes such as river discharge (Rohling and Bigg, 1998; Rosenthal et al., 2000; Weldeab et al., 2007). Quantitative assessments of past sea surface salinities (SSS) may be erroneous, as the relationship between $\delta^{18}O_{sw}$ and salinity varies in space and most likely time too (Rohling and Bigg, 1998). It likely changed due to variable freshwater contributions, for instance, from river discharge or meltwater flux (Rohling and Bigg, 1998).

Weldeab et al. (2007) applied Ba/Ca in planktonic foraminifera as an independent tool for quantitative reconstructions of salinity near rivers. The Ba/Ca ratio of river water is an order of magnitude higher than that of seawater (Weldeab et al., 2007; Schmidt and Lynch-Stieglitz, 2011; Hönisch et al., 2011). Hence, high foraminiferal Ba/Ca ratios provide evidence of increased continental run-off due to precipitation changes in the hinterland (Bahr et al., 2018; Hoffmann et al., 2014; Hall and Chan, 2004). Ba/Ca is apparently not influenced by temperature changes (<7 °C), but its applicability is limited to regions close to river mouths (Weldeab et al., 2007; Bahr et al., 2013). Another theoretical and indirect approach to reconstruct salinity changes is based on the correlation of stable oxygen isotopes ($\delta^{18}O$) and stable hydrogen isotope ratios (δD) between surface waters and the hydrological cycle (Schouten et al., 2006; Rohling, 2007). However, these different ratios must be measured on different archives such as foraminifera calcite ($\delta^{18}O$) and alkenones (δD) and such salinity reconstructions are inherently limited to an error of one salinity unit (Rohling, 2007).

Because of the limitations in current approaches for the quantitative reconstruction of past salinities, the search for a direct salinity proxy is ongoing. Recent culture experiments demonstrated a significant positive correlation between seawater salinity and Na/Ca in foraminiferal calcite of the intertidal benthic foraminifer *Ammonia tepida* (Wit et al., 2013) and the planktonic species *Globigerinoides ruber* (pink) (Allen et al., 2016). A field calibration study of living specimens (*G. ruber* (white) and *Trilobatus sacculifer*) from the Red Sea confirms the positive correlation between Na/Ca_{foram} and salinity, although measured Na/Ca ratios were much higher compared to the culturing studies (Mezger et al., 2016).

Hence, further studies are essential to assess previous results and to understand the main controlling factors on foraminiferal Na incorporation. It is important to know how certain species react to extreme environmental changes and what their tolerance limits are to confirm the robustness of Na/Ca as a salinity proxy over a broad salinity range. Here, we present electron microprobe measurements on *Trilobatus sacculifer*, which were cultured over a salinity range between 26 and 45 (Bijma et al., 1990; Nürnberg et al., 1996). Salinity ranges used in previous culture studies were somewhat limited, with all specimens cultured above a salinity of 30 and below 40 (Wit et al. (2013): S 30–39; Allen et al. (2016): S 33–40). The high spatial resolution microprobe technique used here provides further insights into the inter- and intra-specimen variability of Na/Ca and potential mechanisms involved in Na incorporation. Temperature experiments with cultured *T. sacculifer* grown at temperatures from 19.5 °C to 29.5 °C and constant salinity (Bijma et al., 1990; Nürnberg et al., 1996) were performed to test a potential temperature effect on Na incorporation, as previously suggested by Allen et al. (2016) and Mezger et al. (2016).

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In addition, early diagenetic processes potentially influencing the geochemical signature of foraminiferal calcite while settling through the water column and being buried in deep-sea sediments (Elderfield and Ganssen, 2000; Regenberg et al., 2006), are assessed by directly comparing results from the culture experiments to Na/Ca values for the same species, collected from Caribbean and Gulf of Guinea surface sediments.

2 Material and Methods

2.1 Foraminiferal species studied

The planktonic foraminifer *Trilobatus sacculifer* is a spinose, symbiont-bearing species and its chemical composition (e.g. Mg/Ca, Nürnberg et al., 1996; $\delta^{18}O$, Elderfield and Ganssen, 2000) is widely used in paleoceanography to reconstruct environmental conditions at the ocean's surface. Here, we use the new genus name *Trilobatus* instead of *Globigerinoides* following Spezzaferri et al. (2015), since the latter genus is polyphyletic in its traditional perception based on the presence of supplementary apertures on the spiral side. The species *T. sacculifer* and *T. trilobatus* are genetically identical. They depict different morphotypes that developed during certain ontogenetic stages. Therefore, they were not considered separately in the present study.

In the Caribbean, *T. sacculifer* preferentially inhabits water depths between 10–30 m (Jones, 1968; Hemleben et al., 1987) and is present throughout the year (Schmuker and Schiebel, 2002; Lin et al., 2004). The averaged depth habitat of this species is between 0–40 m in the Caribbean, with highest abundances from 0–20 m (Schmuker and Schiebel, 2002). In the eastern tropical Atlantic, *T. sacculifer* calcifies ontogenetically in the mixed layer at 0–30 m, and slightly deeper in the western tropical Atlantic (0–80 m) within the photic zone (Ravelo and Fairbanks, 1992; Steph et al., 2009). In the subtropical eastern North Atlantic, *T. sacculifer* is most abundant between ~30 and 60 m (Rebotim et al., 2017). Prior to gametogenesis at the end of its life cycle, *T. sacculifer* descends through the water column and precipitates gametogenic calcite after discarding spines, a process related to reproduction (Bé, 1980; Bijma et al., 1990; Bijma and Hemleben, 1994). The formation of a final sac-like chamber is a sign that gametogenesis is impending within 24 hours (Bé, 1980, Hemleben et al., 1989; Erez, 2003).

Since *T. sacculifer* preferentially calcifies in the mixed layer, an averaged depth habitat of 30 m is used for this study. Accordingly, annual salinity and temperature values of 0–30 m water depth were taken from the World Ocean Atlas (WOA) 2013 (Zweng et al., 2013; Locarnini et al., 2013; Schlitzer, 2015) and related to geochemical results of this study.

2.2 Culture experiments

In the following, two sets of experiments are described. For both, specimens of the planktonic foraminiferal species *T. sacculifer* (originally named as *G. sacculifer* in Bijma et al., 1990 and Nürnberg et al., 1996) were collected by SCUBA divers at 3–8 m water depth 1–2 miles off the south coast of Curaçao (see Bijma et al., 1990) and off the west coast of

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Barbados (see Hemleben et al., 1987), respectively. For salinity experiments, the specimens were grown in filtered seawater from the sampling site at salinities of 23, 26, 41, 44 and 45 (abbreviated as S 23–45 below). The different salinities were achieved by evaporating natural seawater at 50 °C or diluting with distilled water. The temperature was held constant at 26.5 °C during salinity experiments. Detailed information on culture protocols and experiments is given in Bijma et al. (1990) and Nürnberg et al. (1996).

For the temperature experiment, specimens were grown in unfiltered seawater from the sampling site at temperatures of 19.5 °, 23.5 °, 26.5 ° to 29.5 °C (all ±0.25 °C), while keeping salinity changes to a minimum (Hemleben et al., 1987). The experiments were run during different seasons between 1980 through 1984 and because salinity varied in the winter and summer season between ~36 to ~31 at the sampling sites, caused by Orinoco and Amazon freshwater discharges, the temperature experiments were divided into 33 salinity (S 33) and 36 salinity (S 36) groups (see Hemleben et al., 1987 for more details). The salinity increased slightly by 0.5–0.8 during the 29.5 °C experiment caused by evaporation in the water bath (Nürnberg et al., 1996). Electron microprobe measurements were carried out on newly grown chambers at defined salinity and temperature conditions. The final, newly grown chamber, the penultimate, and prior chambers are labeled as F (for final), F-1, F-2, F-3 and so forth.

2.3 Surface sediment samples

Caribbean

Foraminiferal tests collected from Caribbean surface sediment samples were used to gain information about the natural interspecific variation of Na/Ca in an open ocean setting with only minor annual changes in salinity and temperature (Figure 1; Table 1). Undisturbed surface sediments were retrieved by the multicorer (MUC) during RV *SONNE* cruise SO164 (Nürnberg et al., 2003). Sample locations are shown in Figure 1a and Table 1. Planktonic foraminifera were selected from the uppermost sediment layer (0–1 cm) and handpicked from the 315–400 µm size fraction. The ages of the surface sediments are classified as late Holocene, and are most likely younger than 2000–3000 years (AMS^{14}C ages before present) (Regenberg et al., 2009; Table 1). During this time period, Caribbean sea surface temperatures (SST) varied by ±1–2 °C at most (Keigwin, 1996; Watanabe et al., 2001; Haase-Schramm et al., 2003).

Surface waters at the sampling locations are composed of Caribbean Surface Water (CSW), characterized by lower salinities throughout the year (S ~33.7–36.3) than the underlying Subtropical Underwater mass at depths of 100–300 m, reaching a salinity maximum of 36.8 (Corredor and Morell, 2001; Schmuker and Schiebel, 2002; Haase-Schramm et al., 2003). The CSW is mainly fed by trade wind driven water masses of the tropical and subtropical Atlantic, entering the NE Caribbean trough the Lesser Antilles (Gordon, 1967). Moreover, the CSW is seasonally influenced by local precipitation and freshwater input from the Orinoco and Amazon rivers in response to shifts of the Intertropical Convergence Zone (Busalacchi and Picaut, 1983; Gordon, 1967). In spite of seasonal variations, the annual sea surface salinity varies only marginally across the

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Caribbean between 35.1 and 36.3 (±0.4) at 30 m depth (Schlitzer, 2015; Zweng et al., 2013, Figure 1c). Annual SST varies between 26.8 °C and 27.7 °C (Locarnini et al., 2013; Figure 1d).

Gulf of Guinea

In addition to the Caribbean samples, surface sediments were chosen from six core locations (GIK16860 to GIK16808) in the Gulf of Guinea, located in the eastern equatorial Atlantic (Figure 1b-d; Table 1). The surface sediments were retrieved by giant box corers from January to February 1988 during RV *METEOR* cruise M6–5 (Lutze et al., 1988; Altenbach et al., 2003). Foraminiferal samples were taken from the uppermost sediment layer (0–1 cm). For geochemical analyses, specimens of *T. sacculifer* were handpicked from the 300–400 µm size fraction. Radiocarbon ages are not available for these surface sediments, but the study of Regenberg et al. (2009) estimates ages of 2430–2730 ±40–50 years BP for E-Atlantic surface sediments proximal to the Gulf of Guinea.

The Gulf of Guinea is influenced by high rates of monsoon-controlled precipitation and large freshwater discharges from the adjacent Sanaga and Niger rivers, with highest input during June to September (Altenbach et al., 2003; Weldeab et al., 2007). The annual SSS varies from east to west at the surface and with increasing water depth (0–60 m) from ~32 to 35.9 (Schlitzer et al., 2015; Zweng et al., 2013; Figure 1c). The annual SST varies around 27.6 °C (±0.6 °C) and decreases with depth to ~19 °C at 60 m (Schlitzer et al., 2015; Locarnini et al., 2013; Figure 1d). To avoid possible calcite dissolution effects, we only chose sampling sites where $\Delta[CO_3^{2-}]$, defined as the difference between the in situ carbonate ion concentration ($[CO_3^{2-}]$) and $[CO_3^{2-}]$ at calcite saturation, is >30 µmol kg^{-1} in bottom waters. Mg/Ca_{foram} is stable at supersaturated bottom waters $\Delta[CO_3^{2-}]$ >40 µmol kg^{-1} (Regenberg et al., 2006; 2014). For instance, foraminiferal Mg/Ca appears unaffected by dissolution above these concentrations (Regenberg et al., 2006; 2014). In the Caribbean and the tropical Atlantic Ocean Mg/Ca_{foram} ratios start decreasing at a critical calcite saturation level of bottom water $\Delta[CO_3^{2-}]$ between ~18–26 µmol kg^{-1}, i.e. below ~2500–3000 m, by 0.5–0.8 mmol mol^{-1} per km (Regenberg et al., 2006; 2009).

2.4 Sample preparation and electron microprobe analysis of culture experiments

Three individual foraminifera per salinity experiment and five individual foraminifera for every temperature experiment were selected from the same size fraction (250–355 µm). Only specimens without a sac-like final chamber were chosen for geochemical analysis to avoid a potential impact from other factors than those targeted here. Newly grown chambers under defined laboratory conditions were compared to chambers grown in situ before placing them in culture (Bijma and Hemleben, 1994). For the electron microprobe analyses (EPMA), all cultured specimens were mounted in epoxy resin (Nürnberg et al., 1996). To expose a fresh and planar surface of test cross sections, the sample mounts were polished with diamond polishing pastes with a 1 µm grain size in the last step. The samples were covered by a 20 nm carbon coating to reduce charging and hence increase the spatial resolution of the obtained maps.

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The measurements were performed on a JEOL JXA 8200 electron microprobe at GEOMAR. In order to map and quantify Na distribution patterns in both single chambers and chamber wall profiles of *T. sacculifer*, quantitative wavelength-dispersive spectrometry (WDS) was applied. Na/Ca ratios were derived from elemental maps, reaching a higher spatial resolution than point measurements (Figure 2). Na/Ca ratios of newly grown calcite were derived from at least 6 single maps along the inner (non-gametogenic) part of the relevant chamber, varying in size between 5 µm x 5 µm and 10 µm x 20 µm (Figure 2). Calcium intensities (wt %) deviating more than 10 % from the maximum Ca intensity within one map were discarded to make sure that measurements on pores or cracks within the shell were excluded. Further to avoid a mixed signal, maps with accumulated higher Na concentrations (±2σ) at calcite spines and spine bases were not integrated into the calibration as well (referring to Branson et al., 2016). Every pixel within a map is 1 µm in diameter and has an element intensity. Pixels were averaged for the entire map (Figure 2). The maps were generated with a focused electron beam, adjusted with an acceleration voltage of 15 kV, a beam current of 80 nA, a spot diameter and step size of 1 µm. The dwell time was set at 50 ms per measurement with 5 accumulations, which implies a total measurement time of 250 ms on the peak position. Na and Ca intensities were measured with the crystals TAPH (Kα) and PETH (Kα). The background intensities were measured separately at almost the same sample location with exactly the same settings and 250 ms accumulation time.

Absolute Na/Ca ratios were quantified using a constant calibration factor for each element after subtracting the background intensities (I_{back}) from the total element intensities (I_{total}). The obtained net intensities were compared to known concentrations in wt % from referenced materials (Table 2). Measurements on standard materials were performed with 10 µm x 10 µm maps (number of pixels: n = 100). The precision of the element analysis and the uncertainty between single measurements is given by the relative standard deviation of averaged element maps (RSD = (standard deviation (1σ) / mean value) · 100) in %, which is less than 5 % for used standard materials (Table 2). To check for matrix effects, the natural modern coral in house standard A2 (Ohde et al., 2003) with a similar elemental composition to foraminiferal calcite was also measured. The accuracy of analyses is expressed as the relative error in %, which represents the deviation between the measured and the reference values (Table 2).

The intra-test variability is expressed as RSD in % and is calculated from averaged Na/Ca maps within chamber wall cross sections of one foraminifer. The inter-test variability (RSD in %) is derived from mean Na/Ca values of one foraminifer and exhibit differences between specimens within the same experiment. Pearson's correlation coefficient R describes within the range of −1 to 1 the correlation between trace elements and the experimental settings, here salinity and temperature. A positive correlation occurs if R is >0 and a negative correlation is displayed when R <0. The appending *p*-value indicates its significance. If *p* is less than 0.05 (5 %) the results are statistically significant. The Na/Ca results for every culture experiment were tested for normality with the Shapiro-Wilk test and corresponding *p*-values (Table A1). If *p* is less or equal to 0.05 (95 % confidence interval), the data are not normally distributed. The range and distribution of averaged Na/Ca maps for each salinity and temperature experiment is shown as box and whisker plots, indicated by the first and third quartiles (Figure 3). The horizontal lines inside the box represent the mean and median. Minimum and maximum values are shown by

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vertical lines (whiskers) outside the box. All Na/Ca values of culture experiments are presented with the standard error of the mean (SEM = (standard deviation σ / √n)).

2.5 ICP–OES sample preparation and analysis of surface sediment samples

Traditional measurements of the elemental composition of foraminiferal calcite employ chemical cleaning procedures as pioneered by Boyle (1981). These were modified for Na/Ca after Barker et al. (2003) as follows. Each sample consisting of 25–35 specimens from the same size fraction (Caribbean: 315–400 µm; Gulf of Guinea: 300–400 µm) were cracked under a binocular and placed in acid cleaned PP micro-centrifuge tubes before being rinsed under ultrasonic agitation with ultrapure water three times, ethanol and again ultrapure water, to remove clays. To eliminate metal oxides in the reductive cleaning step, a buffered solution of hydrous hydrazine and ammonium citrate was added to the samples and reacted in a hot (90 °C) water bath for 30 minutes. The samples were thoroughly rinsed to removed these chemicals with ultrapure water before being transferred to fresh acid cleaned tubes. Residual organic matter was subsequently removed by a 1 % H_2O_2 solution, kept again for 20 min in a hot water bath and rinsed three times with ultrapure water. A final leaching step quickly rinsed with 0.001 M HNO_3 before the samples were finally rinsed once more with ultrapure water. The remaining water was removed and samples were then stored until being dissolved and diluted right before the measurements in 500 µL 0.075 M HNO_3.

The elemental analyses were performed simultaneously with a VARIAN 720 Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP–OES) at GEOMAR. The machine is equipped with a cooled cyclonic spray chamber combined with a microconcentric nebulizer (200 µL min^{-1} sample uptake), optimised for signal stability. The most intense element lines without interferences were chosen for elemental analysis and these wavelengths were Ca 70.60 nm, Mg 279.55 nm and Na 589.59 nm. The ECRM 752-1 is commonly used for foraminiferal Mg/Ca analysis with with a reference value of 3.762 mmol mol^{-1} (Greaves et al., 2008). Hence, the ECRM 752–1 was measured every 10 samples (RSD: 0.06 %) to correct for analytical drift of the instrument and to gain reliable results. As the ECRM 752-1 standard is not certified for Na, we additionally measured the coral reference material JCp–1 (Okai et al., 2002). Referenced Na/Ca values of JCp–1 are 19.79 mmol mol^{-1} and vary during our ICP–OES measurements by ±0.51 mmol/mol from references. The JCp–1 was measured every 10 samples, with RSD of 0.43 % between measurements. Every sample solution was measured 5 times and averaged to one Na/Ca value with RSD of 0.31 % for Na and 0.22 % for Ca.

3 Results

3.1 Sodium incorporation into foraminiferal calcite

Salinity experiments

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Averaged Na/Ca values for the salinity experiments are presented in Table 3. Newly grown chambers of *T. sacculifer* have, on average, Na/Ca values between 3.86 and 6.40 mmol mol^{-1} over a range of 19 salinity units. Foraminiferal Na/Ca values correlate positively with sea surface salinity: $Na/Ca_{foram} = 0.97 + 0.115 \cdot Salinity$ ($R = 0.97$, $p < 0.005$) and increase by 2.25 % per salinity unit, corresponding to an absolute Na/Ca value of 0.12 mmol mol^{-1} (Figure 4). Between salinities of 26 to 36, there is a marked increase in Na/Ca_{foram} by 1–1.25 mmol mol^{-1}, based on the lowest and highest Na/Ca value of each experiment. The smallest possible range is detected between salinities of 33 to 36 where Na/Ca values increase by ~0.5 mmol mol^{-1}, which is 0.1 mmol mol^{-1} greater than the 95 % confidence interval.

The inter-test Na/Ca variability (RSD in %) ranges from 5.5 % to 7.7 % (±0.22–0.29 mmol mol^{-1}) between single foraminifera from the same salinity experiment (Table B1), which is larger than the analytical error of the electron microprobe (RSD ≤0.2 %–1.2 %, Table 2). The intra-test Na/Ca variability (between different maps on one chamber) is twice as large as the inter-test variability and varies between 7.4 % and 13.2% (±0.39–0.69 mmol mol^{-1}, Table B1). The lowest intra- and inter-test variability of around 2 % (0.1 mmol mol^{-1}) is observed in tests, which grew in situ in the open ocean before culturing started. This low variability was observed only for one experiment (culture conditions: 26.5 °C, S 36), representing settings close to the natural habitat of *T. sacculifer*, shown in Figure 3 and 4. Single values of intra- and inter-test variability for each experiment are presented in Table B1.

The largest ranges of Na/Ca within one experiment are observed among foraminifera cultured at salinities >41, presented as box and whisker plots in Figure 3. Intra-test map analyses deviate within chamber cross sections of one foraminifer by ±1.1 mmol mol^{-1}, while inter-test Na/Ca values between foraminifera deviate by ±0.25 mmol mol^{-1}. For the remaining experiments at salinities <41, Na/Ca ratios deviate within the same test by ±0.35–0.65 mmol mol^{-1} and vary among foraminifera by ±0.22–0.55 mmol mol^{-1}, which equals the range of the 95 % confidence interval. Our results from the culture experiment conducted at a salinity of 44 are exceptional: Na/Ca shows a decrease of ~0.5 mmol mol^{-1} compared to the previous experiment at a lower salinity of 41 (Figure 3, 4). According to Nürnberg et al. (1996), all specimens of the S 44-experiment underwent gametogenesis and were enriched in Mg/Ca. Because of differences in elemental composition and calcite structure between gametogenic calcite and ontogenetic calcite (Erez, 2003), the results of the S 44-experiment were hence excluded from the regression shown in Figure 4. Still, even when including foraminifera with gametogenic calcite in our calculations, Na/Ca increases by 2 % per salinity unit ($Na/Ca_{foram} = 1.46 + 0.097 \cdot Salinity$, $R = 0.90$, $p < 0.01$). Na/Ca values measured on chambers (F-3, F-4) grown in situ before sampling and culturing reflect the salinity of ambient seawater in the Caribbean (S ~35.9) and correspond to the results of culture experiments at a salinity of 36 (Zweng et al., 2013; Figure 4). Nonetheless, Na/Ca ratios of chambers grown in situ were not included into the calibration, because the calcification conditions are not exactly known.

Temperature experiments

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Within the 36 salinity group, averaged Na/Ca values of newly grown foraminiferal calcite, precipitated at temperatures from 19.5 to 29.5 °C, range from 3.96 to 5.49 mmol mol^{-1} (Table 4, Figure 5). In the 33 salinity group, averaged Na/Ca vary between 4.10 to 5.78 mmol mol^{-1} within the same temperature interval (Table 5, Figure 5). No significant (p <0.69 (S 36), p <0.14 (S 33)) correlation is found between Na/Ca and temperature for both experiments (Figure 3, 5).

The inter-test variability of Na/Ca (between single foraminifera of the same experiment), expressed as RSD in %, varies from 7.7–9.8 % (S 36) and 3.2–11.5 % (S 33), corresponding to absolute values of ±0.41–0.57 mmol mol^{-1} (S 36) and ±0.15–0.6 mmol mol^{-1} (S 33). Na/Ca values vary within newly formed chambers of one test between 1.8–16.2 % (S 36; ±0.09–0.85 mmol mol^{-1}) and 3.6–10.6 % (S 33; ±0.18–0.55 mmol mol^{-1}). It is interesting to note that the smallest intra-test variation of 1.8 % is observed for treatments close to the natural habitat conditions of *T. sacculifer* (S 36, 26.5 °C). The inter- and intra-test variability of Na/Ca for the different treatments is shown in Table B1. The box and whisker plots demonstrate no trend of Na/Ca with temperature while salinity remained constant at S 33 and S 36 (Figure 3). In total, the Na/Ca intra-test variability between maps within a single wall cross section is less than half during all temperature experiments compared to specimens of each salinity experiments (Figure 3, Table B1).

Surface sediments

The ICP–OES–derived Na/Ca values of *T. sacculifer* from both the Caribbean and Gulf of Guinea surface sediments correspond to the results from single map analyses (electron microprobe) of chamber cross sections from cultured foraminifera (Figure 4, 6). All surface sediment-related data are provided in Table 1. Maximum Na/Ca values (5.67 ±0.23 mmol mol^{-1}) were measured at stations SO164–7–3 and –18–1 northwest of Haiti, showing the highest annual SSS (~36.3) of all sampling sites (Figure 1a, c). Lowest Na/Ca values of 4.71 ±0.21 mmol mol^{-1} were analyzed southwest and southeast of Puerto Rico (stations SO164–22–2 and –23–3), with an annual SSS of ~35.4 (Figure 1a, c). However, some stations are exceptional like SO164-24-3, close to the Puerto Rico stations, where high Na/Ca values of 5.51 mmol mol^{-1} were recorded at apparently low SSS (35.37). Nevertheless, it is still a Na/Ca$_{foram}$ increase of 0.2 mmol mol^{-1} between the latter station and SO164–07–3/18–1 noticeable.

All Caribbean stations exhibit Na/Ca ratios within error of the confidence interval of the Na/Ca-salinity relationship of the culture experiments (Figure 4, 6). This observation is also consistent with Na/Ca values of in situ grown chambers (~27.4 °C, ~S 35.7, WOA13) from those specimens used for culture experiments. The Na/Ca variability of averaged test concentrations (≥30 foraminifera per station) from Caribbean surface sediments varies between 5.5–13 % in RSD (±0.2 to 0.7 mmol mol^{-1}) among stations (Figure 6). This is consistent with the single foraminifera analysis, as the inter-test variability of specimens from salinity (5.5–7.7 %; ±0.2–0.3 mmol mol^{-1}) and temperature (3.2–11.5 %; ±0.15–0.6 mmol mol^{-1}) culture experiments (Figure 3, 4) indicate.

Na/Ca results of the Gulf of Guinea surface sediments, even when taking into account their uncertainty marked by horizontal error bars, lie within the 95 % confidence interval of our culture experiments and have the smallest Na/Ca variability of ≤ 2

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% (≤0.1 mmol mol^{-1}) at all stations (Figure 6). Although the temperature variation is higher (±2.2 °C) than changes in salinity between sampling sites in the Gulf of Guinea, Na/Ca values are in line with results of temperature experiments, demonstrating its limited impact on Na incorporation (Figure 6). The lowest Na/Ca value of 4.77 ±0.03 mmol mol^{-1} in *T. sacculifer* was measured at station GIK 16865-1 close to the Niger and Sanaga river mouths with SSS of 35 at 30 m water depth (Figure 1b, c).

4 Discussion

4.1 Inter- and intra-test variability

The inter- and intra-test element distribution revealed by high-resolution electron microprobe mapping provides additional information about shell Na/Ca composition and heterogeneity, which is fundamental for proxy development. In this study, we explore the variability (expressed as RSD in %) from averaged Na/Ca maps within chamber wall cross sections of one foraminifer and among individual specimens. From our culture experiments the Na/Ca inter- and intra-test variability varies between 3 to 16 % (Table B1). This is in line with previous studies, reporting an inter-test variability of 13–16 % (RSD), measured by laser ablation ICP-MS (Wit et al., 2013; Mezger et al., 2016).

Compared to the Mg/Ca variability (≥30 %) observed in symbiont-bearing foraminiferal species, the Na/Ca inter-test variability of *T. sacculifer* is rather low, half that determined for Mg/Ca (Sadekov et al., 2005; Dueñas-Bohórquez et al., 2011). Previous studies on (trace) metal distribution in various species of planktonic foraminifera showed for instance Mg banding in the chambers, which was explained to be the result of differential Mg-incorporation during biomineralization (Erez, 2003; Eggins et al., 2003; Sadekov et al., 2005; Dueñas-Bohórquez et al., 2011; Spero et al., 2015). The large Mg/Ca variability is primarily due to prominent Mg/Ca banding, alternating in high- and low Mg-calcite bands within chamber walls and different portions of primary and secondary calcite (together called ontogenetic calcite), with Mg being enriched in the primary calcite (Erez, 2003; Sadekov et al., 2005; Sadekov et al., 2008). Potential factors controlling Mg/Ca ratios in banding are changes in calcification temperatures, seawater pH or carbonate ion concentrations and diurnal day/night cycles (Eggins et al., 2004; Davis et al., 2017; Fehrenbacher et al., 2017).

Transferred to our study, higher Na/Ca intra-test variations between maps within a single wall cross sections of each salinity experiment compared to specimens of temperature experiments may occur due to a higher stress level of foraminiferal organisms in culture. This hypothesis is supported by observations of the lowest Na/Ca range in those tests, which grew at culture settings close to the natural habitat of *T. sacculifer* (26.5 °C, S 36) (Figure 3, 4). The biological response of foraminifera to environmental stress could be revealed by the inhibition of chamber formation or gametogenesis (Bijma et al., 1990), as inferred by our cultured foraminifera at different salinities (Nürnberg et al., 1996). Only within one experiment at salinities of 41, foraminifera precipitated more than 2 to 3 new chambers during culture, while just one additional chamber was observed at salinities below and above (Hemleben et al., 1987; Bijma et al., 1990; Nürnberg et al., 1996). In comparison, foraminifera were able to precipitate 3 to 4 new chambers in all temperature experiments.

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315 At the end of their lifecycle foraminifera precipitate more or less gametogenic (GAM) calcite at greater depth, which should be lower in Mg, primary driven by temperature changes (Nürnberg et al., 1996; Erez, 2003; Sadekov et al., 2005). The rate of gametogenesis in the culture experiments was significantly influenced by salinity. At salinities above 41, around 40 % of foraminifera successfully underwent gametogenesis, as shown in culture experiments of Bijma et al. (1990). However, Nürnberg et al. (1996) demonstrated that GAM calcite, when secreted at the same temperatures, is enriched in Mg relative to
320 ontogenetic calcite probably because their biomineralization mechanisms are fundamentally different. Differences between GAM and ontogenetic calcite are also obvious from our study, with reduced Na/Ca values in GAM calcite (Figure 3, 4).

In conclusion, the reduced Na/Ca variability compared to higher Mg/Ca variations implies both, absence or less pronounced Na/Ca banding. This assumption is supported by time-of-flight secondary ionization mass spectrometry (ToF-SIMS) results on the planktonic, symbiont-bearing species *Orbulina universa* (Branson et al., 2016). ToF-SIMS analysis allowing a spatial
325 resolution of 370 nm also showed less Na variability compared to Mg. Only one fine band (<500 nm) enriched in Na was found in all specimens, identified close to the primary organic sheet where the spines of foraminifera arise from (Branson et al., 2016). This is consistent with Erez et al. (2003), who reported higher Na concentrations in proximity of dark layers, most likely the primary organic sheet, of benthic foraminiferal calcite, located between the primary and secondary calcite. Branson et al. (2016) demonstrated that Na is not associated with higher Mg concentrations within tests of *Orbulina universa*.

330 **4.2 Na/Ca as a proxy for salinity**

For *T. sacculifer*, Na incorporation increases significantly (p <0.005) by 2.25 % per salinity unit during culture experiments (Salinity = $((Na/Ca_{foram} - 0.97)/0.115)$) (this study, Figure 4). Within each experiment Na/Ca values are normally distributed (Shapiro-Wilk test, Table A1). Our results are broadly consistent with previous culture studies, which showed a positive correlation between Na/Ca and seawater salinity for the benthic foraminifer *Ammonia tepida*, increasing by 3 % per salinity
335 unit (Wit et al., 2013), and the cultured planktonic species *G. ruber* (pink) (~1 % per salinity unit) (Allen et al., 2016). Allen et al. (2016) observed, however, no significant Na/Ca versus salinity trend in *T. sacculifer* over a salinity range from 33 to 40.

The necessity for establishing species-specific calibrations, similar to foraminiferal Mg/Ca paleothermometry, is demonstrated in Figure 7. As shown in the culture study of Allen et al. (2016), the slope of the Na/Ca *vs.* salinity regression
340 appears steeper for *G. ruber* than for *T. sacculifer* (Fig. 7), suggesting that the sensitivity of Na^+-incorporation in response to salinity change is species-specific, and higher for *G. ruber*. In contrast, no differences were observed in Na/Ca values between *G. ruber* and *T. sacculifer* within the field calibration study from the Red Sea (Mezger et al., 2016). In the latter study Na/Ca values were also markedly higher compared to Wit et al. (2013) and Allen et al. (2016), which the authors explained by the distinct different environmental setting compared to the open ocean (Mezger et al., 2016).

345 With respect to paleosalinity reconstructions, we note that foraminiferal Na/Ca may serve as a direct and reliable proxy for ocean salinities, but has its limitations, as listed further below. We demonstrate for cultured *T. sacculifer* that a change of ±1 salinity unit results in a Na/Ca change of 2.25 %, i.e. ±0.12 mmol mol^{-1} in absolute terms. This definitely exceeds the

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analytical uncertainty of the electron microprobe and ICP–OES (0.2–0.4 % RSD), allowing to resolve past SSS changes of this magnitude.

Although sodium is known to be heterogeneously distributed in foraminiferal calcite (Branson et al., 2016), little small-scale variability was found in our measurements of cultured foraminifera. Since we took care in avoiding parts of the shell with a distinct morphology and (potentially) different chemical composition (e.g. GAM calcite, crust, spines), Na/Ca may be more variable than reported here. However, our electron microprobe analyses show the same range of Na/Ca inhomogeneity between single foraminifera as averaged test concentrations of ≥30 specimens (which all have GAM calcite, albeit various amounts), measured by the ICP–OES of *T. sacculifer* from Caribbean surface sediments (±0.2–0.7 mmol mol^{-1}) (Figure 4, 6). In conclusion of both field studies, the Caribbean and the Gulf of Guinea, changes in salinity of 0.5 to 1 are needed to cause a measurable change in Na/Ca values.

Overall, although differences are observed between species calibrations and high inter- and intra-specimen variability compared to proxy sensitivity, there are still many possibilities for the application of foraminiferal Na/Ca as a proxy for salinity. For instance, large changes in seawater salinity at locations proximal to river mouths in the Gulf of Guinea are reported by Weldeab et al. (2007), who inferred a salinity decrease from ~29 to 24 ±0.4 between 11.46 kyr BP, at the end of YD, to 360 kyr BP. Flower et al. (2004) recorded a peak decay of the Laurentide Ice Sheet and meltwater input to the Gulf of Mexico between 15.2 to 13.0 kyr BP during the Bølling-Ållerød warming, causing a salinity decrease of ~2–4 salinity units. These examples are based, however, on reconstructions of salinity using stable isotopes, based on the modern ice-volume corrected $\delta^{18}O_{ivc\text{-}sw}$–salinity relationship paired with Mg/Ca_{foram} SST, at times both sea water and meltwater inputs are changing, or at times the river input of Ba is varying (Ba/Ca_{foram}–SSS relationship). Clearly, a direct approach for reconstructing salinity would be helpful, even though the resolution would be limited. Instead the addition of Na/Ca_{foram} to a multi-proxy approach could improve the confidence of such reconstructions, as already suggested in Vetter et al. (2017) to combine foraminiferal Mg/Ca and $\delta^{18}O$ with Ba/Ca.

4.3 The reliability of foraminiferal Na/Ca as salinity proxy

Temperature effect on Na incorporation

Before accepting Na/Ca as a robust paleoceanographic tool, factors potentially affecting the primary control of salinity need to be addressed. The statistically insignificant correlation of Na/Ca to temperature changes of cultured *T. sacculifer* in our study (Figure 3 and 5) is in agreement with Allen et al. (2016) (Figure 8). In contrast the Red Sea study of Mezger et al. (2016) showed an apparent correlation between Na/Ca values and temperature for *G. ruber* (R^2 = 0.84, p <0.001) and *T. sacculifer* (R^2 = 0.95, p <0.001) (Figure 8). Because of the co-variability of salinity and temperature in the Red Sea it was, however, not possible to separate effects from each other. But the here shown lack of a temperature effect on Na incorporation in foraminiferal shell carbonate implies that the apparent correlation observed by Mezger et al (2016) was not

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due to temperature. Also a potential impact of the extreme salinity in the Red Sea can be disregarded as the culture study here extended even beyond the very high salinities found in the Red Sea (45 vs 41).

Although *T. sacculifer* prefers higher temperatures (optimum 28 °C) and is mostly absent below 23 °C (Žarić et al., 2005), Na/Ca_{foram} changes are minor in our study within temperature changes (19.5 °–29.5 °C). Additionally, the Na/Ca intra-test variability between maps within a single wall cross section is less than half during almost every temperature experiment compared to specimens of each salinity experiments (Figure 3, Table B1). Hence, there is quite robust support that variations in foraminiferal Na/Ca are not primarily driven by temperature.

Further potential factors controlling Na incorporation

The preparation for analysis is essential as has been exemplarily demonstrated by Rosenthal et al. (2004) and Greaves et al. (2008) for foraminiferal Mg/Ca and its according reference materials. All available Na/Ca data of planktonic and benthic foraminifera are compiled in Figure 7. For the overlapping salinity interval of 30 to 38, averaged Na/Ca ratios of this study are lower by ~1 mmol mol^{-1} than those reported in Wit et al. (2013) and Allen et al. (2016), requiring an explanation (Figure 7). As the *T. sacculifer* specimens from this and from the Allen et al. (2016) study are all from the Caribbean, we assume that the absolute Na/Ca difference could be related to different cleaning techniques applied prior to analyses, which needs further study. The Mezger et al. (2016) field calibration study from the Red Sea also had markedly higher Na/Ca ratios of 7–14 mmol mol^{-1} (±2–6 mmol mol^{-1}) in *G. ruber* (white) and *T. sacculifer* within the salinity range of 37–40. Na/Ca increases by 12 % and 3.9 % per salinity unit in *G. ruber* (w) and *T. sacculifer*, respectively. Mezger et al. (2016) related the extremely high Na/Ca ratios to many co-varying factors in the Red Sea, which are sometimes different from open ocean conditions. Increasing dissolved inorganic carbon (DIC), pH, and calcite saturation state ($\Delta[CO_3^{2-}]$) are likely to cause higher calcite precipitation rates, as observed for cultured benthic foraminifera (Bentov et al., 2009; De Nooijer et al., 2014) and may have caused the high foraminiferal Na/Ca ratios (Mezger et al., 2016). Further, in our study we excluded highly enriched Na zones at spine locations, which are not preserved in the sediment. Our wet chemical Na/Ca analyses (ICP-OES) with >30 specimens per sample match the single foraminiferal analyses by electron microprobe (Figure 4, 6), supporting the quality of our analyses. Compared to previous studies, where entire chambers of living foraminifera with spines included (Allen et al., 2016; Mezger et al., 2016) were measured for elemental analysis, providing a possible mixed Na/Ca signal of spines and shell foraminiferal calcite.

Although statistically not significant (p >0.1, 90 % confidence interval), also Allen et al. (2016) noted a positive trend between Na/Ca in both *G. ruber* (pink) and *T. sacculifer* with increasing $[CO_3^{2-}]$, concentration of DIC, and the pH. Still, they could not see a clear relationship between trace element partitioning and calcification rates in planktonic foraminifera, and hence assumed that calcification rates are not important factors for Na^+ incorporation (Allen et al., 2016).

A major influence on foraminiferal calcification rates and hence, on the uptake of trace elements, is the pH in the micro-environment of foraminiferal tests. The photosynthetic activity of symbionts, depending on nutrient concentrations and light

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intensity actively changes the pH of the ambient micro-environment (Rink et al., 1998; Wolf-Gladrow et al., 1999). Higher photosynthetic rates influencing test geochemistry as has been demonstrated with the boron isotope ratio in symbiont-bearing species like *O. universa* and *T. sacculifer* (Hönisch et al., 2003; De Nooijer et al., 2014). In this regard, averaged Na/Ca values, although within the error when including the standard error of the mean, of *T. sacculifer* from Caribbean surface sediments and those grown in situ before culturing, demonstrate an offset of ~0.3 mmol mol^{-1} above the 95 % confidence interval of cultured specimens (this study, Figure 6). During culture experiments at Barbados and Curaçao, the specimens were exposed to a specific light intensity of 60–70 µmol m^{-2} s^{-1} photons (12 h light/dark cycle), equivalent to underwater light at a depth of 20–30 m off Barbados. The intensity adjustments were fixed and did not change throughout the experiments as in the open ocean (Bijma et al., 1990). We speculate that the photosynthetic activity of symbionts may have been inhibited due to thermal stress during culture experiments. Previous studies reported a high sensitivity of symbionts of benthic and planktonic foraminifera, and the possible loss of chlorophyll *a* ('bleaching') induced by rapid warming during culture experiments (Van Dam et al., 2012) and global warming during the Middle Eocene Climatic Optimum (Edgar et al., 2013). This impacts the geochemical signature of foraminiferal calcite as preserved in sediments (Hönisch et al., 2003; Edgar et al., 2013).

Early diagenetic processes may significantly alter the geochemical signal of fossil foraminifera. It has been widely shown, for example, that foraminiferal Mg/Ca, Cd/Ca or Ba/Ca are affected by calcite dissolution (e.g., Regenberg et al., 2006, 2014; Dekens et al., 2002; McCorkle et al., 1995). Regenberg et al. (2006; 2014) report a decline in foraminiferal Mg/Ca for planktonic species below ~2500–3000 m water depth in the Caribbean, while exceeding the critical calcite saturation level $\Delta[CO_3^{2-}]$ of ~20 µmol kg^{-1} (see section 2.3 for details).

This pattern is applicable for the Caribbean and the tropical Atlantic Ocean, independent of the foraminiferal species (Regenberg et al., 2009; 2014). As the averaged Na/Ca values of foraminifera from Caribbean and Gulf of Guinea surface sediments match (±0.25 mmol mol^{-1}) results of culture experiments (Figure 6, Table 1). Although calcite saturation states in bottom waters are quite different, we suspect that foraminiferal Na/Ca is not influenced by calcite dissolution processes at the sediment-water interface over the last ~3000 years before present This hypothesis is supported by our culture experiments not affected by dissolution, which provide the same slope of regression as surface sediment samples and the same range of inter-test variability (Figure 4, 6). The issue of selective Na^+-removal due to dissolution at greater water depth, nonetheless, needs further investigation.

4.4 Incorporation of Na^+ in foraminiferal calcite

Due to the resemblance of the ionic radii of both Na^+ and Ca^{2+}, Yoshimura et al. (2017) suggested that Na is likely to directly substitute for calcium ions as well as other divalent cations like Mg^{2+} or Sr^{2+} in spite of the difference in charge. In this case two Na^+ ions, paired with anion vacancies (i.e. generated by CO_3^{2-}) as a possible charge compensator, would replace two Ca^{2+} ions (White, 1977) or alternatively one Na^+ ion accompanied by anions as Cl^- or SO_4^{2-}, which induce calcite lattice

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distortions, would replace Ca^{2+} and CO_3^{2-} ions (Yoshimura et al., 2017). However, this incorporation mechanism is only described so far for inorganic precipitation experiments of aragonites in which alkali metals (i.e. Na^+, K^+ or Li^+) directly substitute for Ca^{2+}, whereas alkali metals occupy interstitial positions in inorganic calcite lattice defects due to a different crystal structure (Okumura and Kitano, 1986; Ishikawa and Ichikuni, 1984). This may play a role in the different Na contents of biogenic calcite such as foraminifera (Na: ~1000 ppm, Na/Ca: ~5 mmol mol^{-1}) and aragonite such as corals (Na: ~5000 ppm, Na/Ca: ~20 mmol mol^{-1}) (Yoshimura et al., 2017). Ishikawa and Ichikuni (1984) describe a close positive relationship between Na^+ and salinity in biogenic calcite, albeit this relationship only holds for the low salinity range of 0–10 and disappears above 10. Busenberg and Plummer (1985) report increasing Na/Ca values with calcite growth rate of inorganic calcite, due to the increasing degree of lattice distortions and thus inducing defects in the crystal lattice, promoting Na incorporation.

Notably, in inorganic calcite the partitioning of Na expressed as $D_{Na} = (Na/Ca_{calcite})/(Na/Ca_{ambient\ sw})$ in mmol mol^{-1} varies between $D_{Na} = 0.3–0.8 \cdot 10^{-3}$ (Busenberg and Plummer, 1985; White, 1978), and is therefore markedly higher compared to foraminiferal calcite. In this study, the calculated partition coefficient for Na in *T. sacculifer* is $D_{Na} = 0.11–0.13 \cdot 10^{-3}$, using a $Na/Ca_{seawater}$ = 44.7 mol mol^{-1} as given in Delaney et al. (1985). Their foraminifera were collected from the same sampling site as the cultured specimens of Nürnberg et al. (1996) and this study. Our D_{Na} values agree with previously reported D_{Na}, listed in the following: $D_{Na} = 0.1 \cdot 10^{-3}$, *T. sacculifer*, *G. ruber* (Allen et al., 2016); $D_{Na} = 0.14–0.15 \cdot 10^{-3}$, *T. sacculifer* (Delaney et al., 1985); $D_{Na} = 0.12–0.16 \cdot 10^{-3}$, *A. tepida* (Wit et al., 2013). The field calibration study from the Red Sea of Mezger et al. (2016) found the highest D_{Na} of $0.18–0.25 \cdot 10^{-3}$. The generally two times lower foraminiferal D_{Na} values compared to inorganic calcite suggest a biological control on Na incorporation in foraminiferal calcite, although to a lesser extent than for Mg (De Nooijer et al., 2014). A biological control on Na uptake into foraminiferal calcite is supported by the observation of decreased Na/Ca values at the culture experiment with a high salinity of 44. Such very high salinity conditions might have hampered foraminiferal growth and thereby the ability of the foraminifers to discriminate against Na. Alternatively, previous studies on the same samples proved that specimens at S 44 precipitated gametogenic calcite, which is actually highly enriched in Mg (Nürnberg et al., 1996), and potentially low in Na.

In comparison to foraminiferal D_{Na} the partition coefficient of Mg (D_{Mg}) in planktonic foraminiferal calcite is 5 to 10 times lower than observed for inorganic precipitated calcite (~0.01) (Busenberg and Plummer, 1989; Lea et al., 1999). Additionally Mg/Ca_{foram} increases by 8–10 % per °C, and only 3 %/°C in inorganic calcite, demonstrating a strong biological control and a kinetic and thermodynamic influence on test Mg incorporation (Nürnberg et al., 1996; Lea et al., 1999; Allen et al., 2016). Recent results of Allen et al. (2016) show an increase of D_{Mg} with increasing calcifications rates, but not for D_{Na} of *T. sacculifer* and *G. ruber*.

The salinity dependence of Na incorporation and its insensitivity to temperature affords further examination. Wit et al. (2013) suggested that Na^+ incorporation into foraminiferal calcite is mainly driven by the activity ratio of free Na^+ to free Ca^{2+} in seawater, which is related to their concentrations in seawater and increases with salinity (Ishikawa and Ichikuni,

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1984; Zeebe and Wolf-Gladrow, 2001). In contrast, Na^{+} and Ca^{2+} activities in seawater are only to a limited extent affected by temperature changes, and hence also not the Na incorporation (Ishikawa and Ichikuni, 1984; Delaney et al., 1985; Zeebe and Wolf-Gladrow, 2001). Instead, lower temperatures could cause enhanced solubility of calcium carbonate, resulting in an increase of free Ca^{2+} (Zeebe and Wolf-Gladrow, 2001). This could induce lower Na/Ca ratios in foraminiferal calcite at higher temperatures and would explain the slightly, but insignificant, negative response of Na/Ca_{foram} to temperature.

5 Conclusions

Foraminiferal Na/Ca of cultured *T. sacculifer* correlates positively ($R = 0.97$, $p < 0.005$) with increasing sea surface salinity between 26 to 45 salinity units: $Salinity = ((Na/Ca_{foram} - 0.97)/0.115)$. A strong temperature control on Na incorporation in foraminiferal calcite can be excluded by our data, supporting previous culture studies (Allen et al. (2016). In our experiments we observed a rather low Na/Ca inter- and intra-test variability of 3–16 %, half that determined for Mg/Ca (≥30 %) in symbiont-bearing foraminiferal species (Sadekov et al., 2005; Dueñas-Bohórquez et al., 2011). In conclusion, the reduced Na/Ca variability compared to higher Mg/Ca variations implies both, absence or less pronounced Na/Ca banding. As no relationship between Na partitioning and calcification rates was observed in foraminiferal calcite (Allen et al., 2016), there is quite robust support that variations in Na/Ca_{foram} are primarily driven by salinity.

According to our results, the change of ±1 salinity unit would result in a Na/Ca change in *T. sacculifer* calcite of 2.25 %, or ±0.12 mmol mol^{-1}. This is in accordance with results of *T. sacculifer* from both the Caribbean and the Gulf of Guinea surface sediments. The agreement of surface sediment and culture experiment data suggesting that dissolution processes at the sediment-water interface do not influence foraminiferal Na/Ca. Our new data further support that foraminiferal Na/Ca may serve as a direct and reliable proxy for ocean salinities, but the application is limited to large salinity changes. Salinity changes below <0.5 salinity units would not be detectable, and Na/Ca_{foram} is species specific, as demonstrated by the offset of 1–3 mmol mol^{-1} between various planktonic and benthic species (Wit et al., 2013; Allen et al., 2016). Further improvement and proxy development could be achieved by a multi-proxy approach, i.e. using Na/Ca paired with Mg/Ca_{foram}, $\delta^{18}O_{sw}$ and Ba/Ca, to support the confidence of past salinity reconstructions.

Data availability

All data used in this study are listed in the manuscript and the appendices, but will be additionally uploaded to the online data publisher PANGEA.

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Sample availability

All used sample materials are archived by the GEOMAR Helmholtz Centre for Ocean Research Kiel (contact: dnuernberg@geomar.de).

Author contributions

JB and DN designed this study. Data analyses and preparation of the manuscript was done by JB, assisted by DN. Culture experiments described in this study were initially carried out by JBj. Measurements of Gulf of Guinea foraminiferal samples were accomplished by SN. GJR, EM, LDN, EH, JS, JBj and MK contributed to data interpretation and significantly improved the manuscript with intense discussions and text iterations.

Competing interests

The authors declare that they have no conflict of interest.

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Tables

Table 1. Na/Ca values of *T. sacculifer* from surface sediment samples, obtained from different core locations in the Gulf of Guinea[a] (Lutz et al., 1988) and the Caribbean[b] (Nürnberg et al., 2003). The salinity and temperature data present averaged annual values at 30 m water depth ±30 m (WOA13), which is shown as the standard error of the mean (SEM). Na/Ca ratios[a,b] (±SEM) were measured by an ICP–OES. Radiocarbon (AMS^{14}C) ages before present (BP) are published in Regenberg et al. (2009).

Cruise Station	Latitude (°N)	Longitude (°E)	Water depth (m)	Surface (cm)	^{14}C Age years BP	Salinity	Temperature (°C)	**Na/Ca$_{average}$ (mmol/mol)**
Gulf of Guinea (M6−5)[a]								
GIK 16808−2	3° 48.6'	2° 51.5'	2234	0−1		35.31 ±0.17	25.20 ±1.32	4.92 ±0.09
GIK 16860−1	3° 43.7'	−6° 29.9'	202	0−1		34.96 ±0.62	25.42 ±1.35	5.01 ±0.07
GIK 16864−1	3° 09.3'	−6° 17.1'	1495	0−1		34.98 ±0.54	25.72 ±1.34	4.92 ±0.10
GIK 16865−1	2° 40.3'	−6° 03.3'	2492	0−1		35.01 ±0.45	25.87 ±1.32	4.77 ±0.03
GIK 16868−2	−0° 13.7'	−6° 01.1'	1284	0−1		35.51 ±0.31	23.65 ±1.21	4.92 ±0.14
GIK 16869−1	−0° 12.7'	−6° 00.8'	1837	0−1		35.49 ±0.32	23.90 ±1.23	5.12 ±0.12
Caribbean Sea (SO164)[b]								
01−3	13° 50.195'	74° 09.028'	4026	0−1		35.97 ±0.05	27.23 ±0.10	4.81 ±0.28
02−3	15° 18.29'	72° 47.06'	2977	0−1	2205 ±25	35.71 ±0.05	27.45 ±0.05	5.24 ±0.30
03−3	16° 32.40'	72° 12.31'	2744	0−1		35.63 ±0.06	27.50 ±0.04	5.54 ±0.27
04−2	17° 16.38'	71° 39.09'	1013	0−1		35.50 ±0.06	27.53 ±0.03	5.32 ±0.37
07−3	21° 19.46'	74° 08.76'	2722	0−1	720 ±35	36.37 ±0.02	26.96 ±0.15	5.67 ±0.17
18−1	21° 13.61'	74° 21.0'	1629	0−1	1350 ±25	36.27 ±0.03	27.09 ±0.15	5.68 ±0.30
20−2	16° 45.49'	71° 29.22'	3357	0−1		35.57 ±0.05	27.49 ±0.05	5.13 ±0.26
21−3	16° 06.0'	70° 30.0'	3995	0−1		35.63 ±0.06	27.49 ±0.06	5.06 ±0.30
22−2	15° 24.0'	68° 12.0'	4506	0−1		35.60 ±0.08	27.61 ±0.07	4.79 ±0.18
23−3	15° 34.01'	65° 08.09'	4328	0−1		35.42 ±0.13	27.60 ±0.08	4.64 ±0.25
24−3	14° 11.89'	63° 25.43'	1545	0−1		35.37 ±0.15	27.55 ±0.12	5.51 ±0.33
25−3	14° 41.25'	59° 44.48'	2720	0−1	1915 ±30	35.45 ±0.16	27.45 ±0.10	5.32 ±0.25
48−2	15° 57.02'	60° 55.0'	1286	0−1		35.59 ±0.14	27.34 ±0.09	5.40 ±0.56
50−3	15° 21.25'	59° 16.94'	4002	0−1		35.55 ±0.15	27.39 ±0.10	5.19 ±0.17

[a] Foraminifera were handpicked from the 300–400 µm size fraction. Each Na/Ca value derives from the average of 25–30 specimens and is the mean of 3–5 repeated ICP–OES measurements.
[b] Foraminifera were selected from the 315–400 µm size fraction. Every ICP–OES measurement was repeated 5 times. One sample contains ≥30 specimen.

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Table 2. Reference materials used for electron microprobe analysis. Precision (Pr) and accuracy (Ac) is expressed as the relative standard deviation (RSD) and the relative error in %. Measurements were performed with 10 µm x 10 µm maps (number of pixels: n = 100).

Standard	Reference	Ca (wt%)	Pr (%)	Ac (%)	Na (wt%)	Pr (%)	Ac (%)
Calcite (USNM 136321)	Jarosewich, 2002	40.11	0.27	0.02	–	–	–
Dolomite (USNM 10057)	Jarosewich, 2002	21.85	1.97	0.55	–	–	–
Albite (131705, Natural Plagioclase Feldspar)	Amelia Co., Virginia, USA	–	–	–	8.71	0.17	-0.95
Glass, Basalt (USNM 111240 VG-2)	Jarosewich, 2002	7.95	2.16	4.75	1.94	1.2	-11.66
Modern Coral A2	Ohde et al., 2003	37.84	0.83	2.56	0.47	8.73	-25.78

Table 3. Individual Na/Ca values of cultured *T. sacculifer* under variable salinity and constant temperature (26.5 °C ±0.25 °C), measured by the electron microprobe. Each value results from maps, ranging in size from 5 to 20 µm, on inner chamber wall cross sections of newly grown calcite (F, F-1) during the experiments. Each pixel represents 1 µm x 1 µm. Averaged values comprise all Na/Ca values of one single foraminifer. Sample labels correspond to those used in Nürnberg et al. (1996). Chambers grown in situ (F-3 and F-4) are labelled and averaged separately from those grown in the natural environment before culturing. Annual salinity data were taken from WOA13 (Zweng et al., 2013). Individual and averaged Na/Ca uncertainty values are based on ±standard error of the mean (σ/√n).

Sample #	Salinity	Pixel n	$Na/Ca_{individual}$ (mmol mol^{-1})	$Na/Ca_{average}$ (mmol mol^{-1})	Sample #	Salinity	Pixel n	$Na/Ca_{individual}$ (mmol mol^{-1})	$Na/Ca_{average}$ (mmol mol^{-1})
7912	26	35	4.77 ±0.22	**4.36 ±0.20**	7703	44	21	5.40 ±0.25	
7912	26	25	4.15 ±0.20		7703	44	24	4.62 ±0.22	
7912	26	25	3.73 ±0.25		7703	44	18	3.96 ±0.27	
7912	26	25	4.82 ±0.39		7703	44	15	4.62 ±0.47	
7912	26	72	4.34 ±0.16		7703	44	20	4.53 ±0.40	
					7703	44	19	5.32 ±0.34	
7913	26	144	4.36 ±0.09	**3.96 ±0.14**					
7913	26	24	3.84 ±0.31		7704	44	72	5.51 ±0.19	**5.27 ±0.16**
7913	26	36	4.18 ±0.23		7704	44	43	4.36 ±0.16	
7913	26	33	3.51 ±0.22		7704	44	42	6.50 ±0.33	
7913	26	36	3.27 ±0.16		7704	44	91	5.39 ±0.13	
7913	26	35	3.88 ±0.16		7704	44	21	5.12 ±0.27	
7913	26	20	4.34 ±0.37		7704	44	22	4.83 ±0.24	
7913	26	21	4.28 ±0.24		7704	44	21	4.43 ±0.34	
					7704	44	20	5.45 ±0.34	
7914	26	94	3.81 ±0.16	**3.86 ±0.17**	7704	44	22	5.62 ±0.45	
7914	26	48	4.04 ±0.20		7704	44	48	4.85 ±0.20	
7914	26	47	4.71 ±0.28		7704	44	45	5.64 ±0.21	
7914	26	35	3.56 ±0.20		7704	44	48	5.43 ±0.20	
7914	26	30	4.04 ±0.31		7704	44	105	5.37 ±0.14	
7914	26	34	3.42 ±0.19						
7914	26	25	3.46 ±0.19		8301	45	47	5.91 ±0.25	**6.40 ±0.19**
					8301	45	49	6.15 ±0.25	
8135	41	23	5.97 ±0.33	**5.66 ±0.20**	8301	45	47	6.20 ±0.28	

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8135	41	18	4.97 ±0.33	
8135	41	19	4.74 ±0.48	
8135	41	22	5.61 ±0.42	
8135	41	135	6.18 ±0.16	
8135	41	143	5.01 ±0.10	
8135	41	140	4.83 ±0.09	
8135	41	22	4.24 ±0.32	
8135	41	45	6.33 ±0.25	
8135	41	70	6.46 ±0.24	
8135	41	74	6.20 ±0.21	
8135	41	69	6.12 ±0.30	
8135	41	79	6.48 ±0.29	
8135	41	45	6.04 ±0.22	
8137	41	24	5.04 ±0.25	**5.49 ±0.16**
8137	41	19	4.84 ±0.29	
8137	41	22	5.46 ±0.29	
8137	41	22	4.30 ±0.21	
8137	41	21	5.20 ±0.28	
8137	41	23	5.43 ±0.34	
8137	41	64	5.85 ±0.18	
8137	41	21	5.32 ±0.40	
8137	41	23	5.33 ±0.29	
8137	41	25	6.47 ±0.49	
8137	41	21	5.20 ±0.23	
8137	41	24	6.45 ±0.30	
8137	41	20	6.09 ±0.35	
8137	41	23	5.88 ±0.35	
8138	41	46	4.41 ±0.21	**5.08 ±0.15**
8138	41	46	5.42 ±0.35	
8138	41	49	5.16 ±0.26	
8138	41	49	5.40 ±0.29	
8138	41	48	4.90 ±0.20	
8138	41	49	5.16 ±5.19	
7703	44	19	5.03 ±0.40	**4.83 ±0.18**
7703	44	24	4.60 ±0.33	
7703	44	46	6.06 ±0.24	
7703	44	23	4.72 ±0.26	
7703	44	24	4.23 ±0.25	
8301	45	44	6.71 ±0.31	
8301	45	46	6.02 ±0.20	
8301	45	45	5.90 ±0.21	
8301	45	131	6.39 ±0.13	
8301	45	43	5.62 ±0.21	
8301	45	100	7.55 ±0.13	
8301	45	100	7.68 ±0.18	
8301	45	186	6.84 ±0.12	
8301	45	98	5.87 ±0.17	
In situ grown chambers (F-3, F-4)				
4.1	35.9	219	5.16 ±0.12	**5.27 ±0.12**
4.1	35.9	263	4.96 ±0.08	
4.1	35.9	257	5.06 ±0.08	
4.1	35.9	151	5.53 ±0.14	
4.1	35.9	195	5.73 ±0.10	
4.1	35.9	195	5.18 ±0.09	
2.3	35.9	105	5.49 ±0.14	**5.43 ±0.18**
2.3	35.9	58	5.55 ±0.17	
2.3	35.9	65	5.51 ±0.19	
2.3	35.9	72	5.47 ±0.18	
2.3	35.9	98	5.38 ±0.19	
2.3	35.9	108	5.05 ±0.12	
2.3	35.9	66	5.55 ±0.22	
7704	35.9	109	5.43 ±0.14	**5.48 ±0.04**
7704	35.9	68	5.60 ±0.17	
7704	35.9	99	5.47 ±0.13	
7704	35.9	72	5.51 ±0.15	
7704	35.9	78	5.33 ±0.15	
7704	35.9	96	5.51 ±0.12	
7703	35.9	21	5.40 ±0.25	**5.34 ±0.08**
7703	35.9	24	5.10 ±0.24	
7703	35.9	45	5.16 ±0.18	
7703	35.9	39	5.58 ±0.18	
7703	35.9	62	5.57 ±0.17	
7703	35.9	66	5.25 ±0.17	

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Table 4. Individual Na/Ca values of cultured *T. sacculifer* under variable temperatures and a constant salinity of 36, measured by the electron microprobe. Each value results from maps, ranging in size from 5 to 20 µm, on inner chamber wall cross sections of newly grown calcite (F, F-1) during the experiments. Each pixel represents 1µm x 1 µm. Averaged values comprise all Na/Ca values of one single foraminifer. Sample labels correspond to those used in Nürnberg et al. (1996). Individual and averaged Na/Ca values are based on ±standard error of the mean (σ/√n).

Sample #	T (°C)	Pixel n	$Na/Ca_{individual}$ (mmol mol^{-1})	$Na/Ca_{average}$ (mmol mol^{-1})	Sample #	T (°C)	Pixel n	$Na/Ca_{individual}$ (mmol mol^{-1})	$Na/Ca_{average}$ (mmol mol^{-1})
1.2	19.5	36	4.47 ±0.34	**4.24 ±0.19**	3.1	26.5	46	4.69 ±0.17	**4.62 ±0.15**
1.2	19.5	44	4.64 ±0.43		3.1	26.5	48	4.26 ±0.18	
1.2	19.5	47	3.75 ±0.17		3.1	26.5	48	4.57 ±0.25	
1.2	19.5	48	4.04 ±0.20		3.1	26.5	45	4.97 ±0.22	
1.2	19.5	24	3.49 ±0.24						
1.2	19.5	15	4.11 ±0.35		3.2	26.5	13	4.76 ±0.24	**4.86 ±0.10**
1.2	19.5	19	5.06 ±0.46		3.2	26.5	15	4.54 ±0.37	
1.2	19.5	19	5.17 ±0.28		3.2	26.5	100	4.71 ±0.24	
1.2	19.5	47	4.68 ±0.34		3.2	26.5	100	5.20 ±0.19	
1.2	19.5	48	3.80 ±0.17		3.2	26.5	49	4.32 ±0.17	
1.2	19.5	47	3.68 ±0.22		3.2	26.5	45	4.89 ±0.21	
					3.2	26.5	45	4.91 ±0.16	
1.3	19.5	25	4.43 ±0.30	**4.78 ±0.24**	3.2	26.5	49	4.80 ±0.20	
1.3	19.5	24	4.47 ±0.32		3.2	26.5	48	4.59 ±0.18	
1.3	19.5	24	4.58 ±0.32						
1.3	19.5	22	4.43 ±0.31		3.4	26.5	100	5.40 ±0.20	**5.00 ±0.07**
1.3	19.5	16	4.83 ±0.35		3.4	26.5	100	4.98 ±0.13	
1.3	19.5	15	5.95 ±0.87		3.4	26.5	49	4.72 ±0.22	
					3.4	26.5	45	5.11 ±0.23	
1.4	19.5	19	4.76 ±0.42	**5.09 ±0.20**	3.4	26.5	49	5.09 ±0.24	
1.4	19.5	13	4.07 ±0.56		3.4	26.5	47	4.92 ±0.19	
1.4	19.5	20	5.97 ±0.61		3.4	26.5	49	4.81 ±0.19	
1.4	19.5	22	4.39 ±0.31		3.4	26.5	20	4.89 ±0.32	
1.4	19.5	45	4.68 ±0.17		3.4	26.5	22	5.06 ±0.34	
1.4	19.5	48	5.32 ±0.25						
1.4	19.5	49	5.07 ±0.28		3.5	26.5	112	5.46 ±0.13	**5.49 ±0.05**
1.4	19.5	121	5.08 ±0.12		3.5	26.5	102	5.38 ±0.15	
1.4	19.5	49	5.49 ±0.25		3.5	26.5	75	5.62 ±0.21	
1.4	19.5	22	6.03 ±0.49		3.5	26.5	89	5.51 ±0.14	
2.1	23.5	20	4.25 ±0.25	**4.74 ±0.16**	4.1	29.5	100	4.86 ±0.14	**4.80 ±0.08**
2.1	23.5	24	4.83 ±0.27		4.1	29.5	48	4.60 ±0.17	
2.1	23.5	23	5.06 ±0.22		4.1	29.5	42	5.11 ±0.25	
2.1	23.5	24	5.06 ±0.23		4.1	29.5	48	4.91 ±0.19	
2.1	23.5	21	4.51 ±0.31		4.1	29.5	49	4.64 ±0.13	
					4.1	29.5	23	4.39 ±0.24	
2.2	23.5	149	5.65 ±0.13	**5.41 ±0.06**	4.1	29.5	25	4.60 ±0.32	
2.2	23.5	100	5.40 ±0.14		4.1	29.5	64	4.97 ±0.15	
2.2	23.5	100	5.15 ±0.11		4.1	29.5	64	4.70 ±0.17	
2.2	23.5	142	5.63 ±0.14		4.1	29.5	104	5.22 ±0.18	
2.2	23.5	178	5.39 ±0.10						
2.2	23.5	149	5.17 ±0.10		4.3	29.5	16	4.06 ±0.35	**3.96 ±0.23**
2.2	23.5	97	5.57 ±0.15		4.3	29.5	19	3.73 ±0.20	

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2.2	23.5	98	5.23 ±0.16		4.3	29.5	17	3.60 ±0.21	
2.2	23.5	99	5.46 ±0.15		4.3	29.5	17	3.62 ±0.22	
					4.3	29.5	23	4.80 ±0.23	
2.3	23.5	25	4.53 ±0.23	**4.99 ± 0.33**					
2.3	23.5	25	4.05 ±0.27		4.4	29.5	25	4.05 ±0.27	**4.26 ±0.12**
2.3	23.5	25	4.45 ±0.17		4.4	29.5	25	3.93 ±0.25	
2.3	23.5	47	6.19 ±0.38		4.4	29.5	25	4.33 ±0.22	
2.3	23.5	49	5.66 ±0.21		4.4	29.5	49	4.26 ±0.23	
2.3	23.5	48	5.05 ±0.16		4.4	29.5	48	3.86 ±0.16	
					4.4	29.5	45	3.94 ±0.18	
2.4	23.5	24	4.52 ±0.29	**4.51 ± 0.37**	4.4	29.5	49	4.34 ±0.15	
2.4	23.5	48	5.03 ±0.23		4.4	29.5	96	4.82 ±0.15	
2.4	23.5	42	4.22 ±0.19		4.4	29.5	104	4.84 ±0.13	
2.4	23.5	44	4.28 ±0.20						

Table 5. Individual Na/Ca values of cultured *T. sacculifer* under variable temperatures and a constant salinity of 33, measured by the electron microprobe. Each value results from maps, ranging in size from 5 to 20 µm, on inner chamber wall cross sections of newly grown calcite (F, F-1) during the experiments. Each pixel represents 1µm x 1 µm. Averaged values comprise all Na/Ca values of one single foraminifer. Sample labels correspond to those used in Nürnberg et al. (1996). Individual and averaged Na/Ca values are based on ±standard error of the mean (σ/√n).

Sample #	T (°C)	Pixel n	$Na/Ca_{individual}$ (mmol mol^{-1})	$Na/Ca_{average}$ (mmol mol^{-1})	Sample #	T (°C)	Pixel n	$Na/Ca_{individual}$ (mmol mol^{-1})	$Na/Ca_{average}$ (mmol mol^{-1})
5.1	19.5	34	5.29 ±0.26	**5.37 ±0.10**	7.1	26.5	23	3.97 ±0.24	
5.1	19.5	42	5.41 ±0.22		7.1	26.5	23	4.46 ±0.38	
5.1	19.5	48	5.64 ±0.19		7.1	26.5	20	4.52 ±0.29	
5.1	19.5	39	5.27 ±0.20		7.1	26.5	20	4.54 ±0.23	
5.1	19.5	33	5.40 ±0.26		7.1	26.5	17	4.48 ±0.28	
5.1	19.5	33	5.65 ±0.26		7.1	26.5	22	4.60 ±0.29	
5.1	19.5	20	4.90 ±0.35		7.1	26.5	24	4.18 ±0.29	
5.2	19.5	63	4.91 ±0.17	**5.13 ±0.15**	7.3	26.5	100	5.00 ±0.18	**5.05 ±0.09**
5.2	19.5	63	4.90 ±0.15		7.3	26.5	47	5.05 ±0.20	
5.2	19.5	100	5.69 ±0.15		7.3	26.5	23	5.23 ±0.26	
5.2	19.5	99	5.62 ±0.16		7.3	26.5	43	4.64 ±0.19	
5.2	19.5	21	4.57 ±0.29		7.3	26.5	85	5.45 ±0.15	
5.2	19.5	21	4.76 ±0.27		7.3	26.5	46	4.81 ±0.19	
5.2	19.5	20	4.56 ±0.21		7.3	26.5	45	5.09 ±0.21	
5.2	19.5	19	5.64 ±0.47		7.3	26.5	44	5.14 ±0.23	
5.2	19.5	16	4.70 ±0.32						
5.2	19.5	17	5.99 ±0.39		7.4	26.5	90	5.24 ±0.15	**5.05 ±0.14**
5.2	19.5	23	5.13 ±0.25		7.4	26.5	41	4.60 ±0.16	
					7.4	26.5	52	4.87 ±0.19	
5.5	19.5	46	4.56 ±0.20	**5.05 ±0.09**	7.4	26.5	46	4.99 ±0.23	
5.5	19.5	37	5.06 ±0.32		7.4	26.5	43	5.04 ±0.19	
5.5	19.5	40	5.35 ±0.20		7.4	26.5	45	4.86 ±0.19	
5.5	19.5	42	5.01 ±0.19		7.4	26.5	96	5.75 ±0.16	

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5.5	19.5	23	5.42 ±0.29						
5.5	19.5	17	5.03 ±0.43		7.5	26.5	19	4.19 ±0.34	**4.61 ±0.20**
5.5	19.5	25	5.07 ±0.32		7.5	26.5	25	4.83 ±0.30	
5.5	19.5	25	4.89 ±0.27		7.5	26.5	14	5.02 ±0.27	
					7.5	26.5	17	4.68 ±0.35	
6.1	23.5	85	5.68 ±0.17	**5.53 ±0.07**	7.5	26.5	17	5.06 ±0.47	
6.1	23.5	92	5.42 ±0.17		7.5	26.5	16	3.85 ±0.32	
6.1	23.5	89	5.73 ±0.17						
6.1	23.5	43	5.13 ±0.20		8.1	29.5	24	4.98 ±0.24	**4.39 ±0.13**
6.1	23.5	24	5.71 ±0.38		8.1	29.5	24	4.12 ±0.27	
6.1	23.5	45	5.46 ±0.18		8.1	29.5	22	4.66 ±0.31	
6.1	23.5	79	5.57 ±0.17		8.1	29.5	24	4.35 ±0.29	
6.1	23.5	98	5.55 ±0.13		8.1	29.5	20	3.90 ±0.20	
					8.1	29.5	18	4.58 ±0.25	
6.2	23.5	54	4.58 ±0.16	**4.53 ±0.12**	8.1	29.5	22	4.52 ±0.26	
6.2	23.5	21	4.74 ±0.24		8.1	29.5	16	4.00 ±0.44	
6.2	23.5	25	4.39 ±0.24						
6.2	23.5	25	4.80 ±0.21		8.3	29.5	15	3.95 ±0.36	**4.10 ±0.13**
6.2	23.5	25	4.27 ±0.24		8.3	29.5	14	3.67 ±0.31	
6.2	23.5	25	4.04 ±0.28		8.3	29.5	17	4.14 ±0.25	
6.2	23.5	24	4.86 ±0.24		8.3	29.5	15	3.96 ±0.23	
					8.3	29.5	14	3.79 ±0.29	
6.3	23.5	18	5.24 ±0.21	**5.16 ±0.21**	8.3	29.5	45	4.13 ±0.19	
6.3	23.5	18	5.10 ±0.28		8.3	29.5	50	3.82 ±0.15	
6.3	23.5	17	4.67 ±0.31		8.3	29.5	45	4.63 ±0.22	
6.3	23.5	21	4.86 ±0.40		8.3	29.5	44	4.78 ±0.17	
6.3	23.5	20	5.92 ±0.39						
					8.4	29.5	47	4.80 ±0.17	**4.43 ±0.14**
6.5	23.5	47	6.05 ±0.24	**5.78 ±0.11**	8.4	29.5	38	4.68 ±0.18	
6.5	23.5	41	5.47 ±0.20		8.4	29.5	35	4.48 ±0.22	
6.5	23.5	42	6.12 ±0.20		8.4	29.5	20	4.04 ±0.23	
6.5	23.5	35	6.02 ±0.22		8.4	29.5	23	4.11 ±0.28	
6.5	23.5	44	5.71 ±0.25		8.4	29.5	17	3.93 ±0.31	
6.5	23.5	24	6.12 ±0.24		8.4	29.5	42	4.33 ±0.38	
6.5	23.5	44	5.17 ±0.22		8.4	29.5	23	5.04 ±0.35	
6.5	23.5	98	6.14 ±0.14						
6.5	23.5	134	5.97 ±0.12		8.5	29.5	61	4.81 ±0.16	**5.31 ±0.21**
6.5	23.5	93	5.25 ±0.12		8.5	29.5	63	5.14 ±0.18	
6.5	23.5	93	5.59 ±0.14		8.5	29.5	59	5.57 ±0.20	
					8.5	29.5	42	5.72 ±0.26	
7.1	26.5	22	3.90 ±0.24	**4.33 ±0.10**					

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Figures

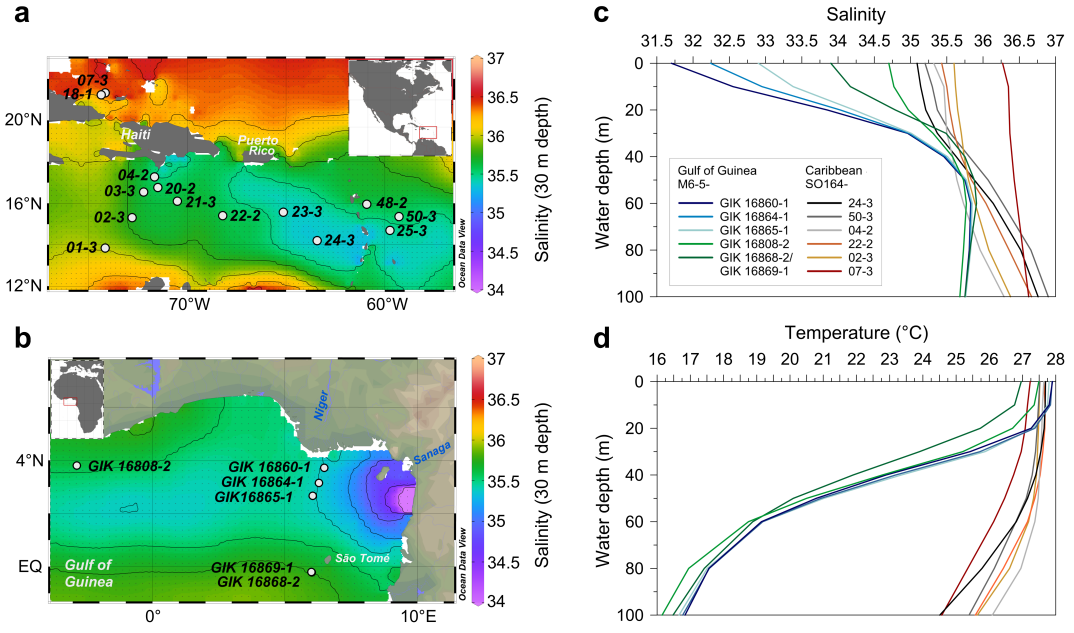

Figure 1. Sediment surface samples used for Na/Ca calibration studies (a-b) and modern hydrography in the study areas (c-d). **(a)** Annual sea surface salinity in the Caribbean at 30 m depth (World Ocean Atlas 2013; Zweng et al., 2013). Surface sediments (white dots) were taken during the RV *SONNE* cruise SO164 (Nürnberg et al., 2003). **(b)** Annual sea surface salinity in the Gulf of Guinea at 30 m depth (World Ocean Atlas 2013; Zweng et al., 2013). Sediment samples (white dots) are from RV *METEOR* cruise M 6–5 (Lutze et al., 1988). Station numbers are indicated (Table 2). Maps were created with Ocean Data View (Schlitzer, 2015). **(c)** Annual range of salinities and **(d)** temperatures from 0 to 100 m water depth in the Caribbean and the Gulf of Guinea (World Ocean Atlas 2013; Zweng et al., 2013; Locarnini et al., 2013). Sample stations are color-coded (see legend). Stations of Caribbean surface sediments, which are not shown, vary within the black and yellow lines. Station SO164–18–1 equals station –07–3. Data are listed in Table 2.

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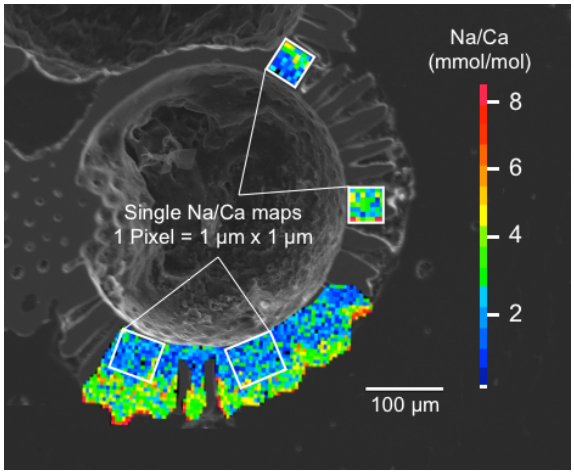

Figure 2. Secondary electron image with overlying maps of Na/Ca values in chamber wall cross sections of cultured *T. sacculifer*, measured by the electron microprobe. This is an example of single maps within one chamber. One pixel is 1 µm x 1 µm and the total map sizes vary from 5 µm to 20 µm, depending on chamber wall's thickness. Averaged Na/Ca maps are used to present results of both salinity and temperature experiments. Na and Ca intensities deviating more than 2σ from maximum intensities within a map, were discarded.

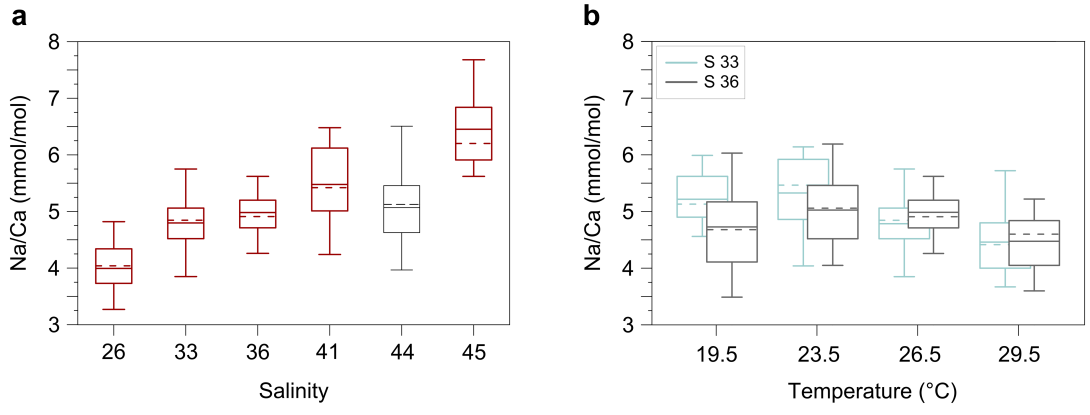

Figure 3. Box and whisker plots of the range and distribution of Na/Ca in newly grown test chambers of *T. sacculifer* during culture experiments. The boxes include all data within the first and third quartile. The horizontal solid line in the centre of each box demonstrates the mean and the dashed line represents the median. Minimum and maximum values are marked by vertical lines (whiskers). Color-coding is similar to Figures 4 and 5. **(a)** Na/Ca within test chambers grown at varying salinities (26–45) and constant temperature (26.5 °C ±0.25 °C). Number of measurements for each experiment from lowest to highest salinity: n = 20, 29, 26, 34, 24, 12. **(b)** Na/Ca within test chambers grown at varying temperatures (19.5 °–29.5 °C) in two salinity groups at either constant salinity of 33 (S 33) or 36 (S 36). Number of measurements for each experiment from lowest to highest temperatures: n (S 33) = 26, 31, 29, 29; n (S 36) = 26, 24, 26, 24.

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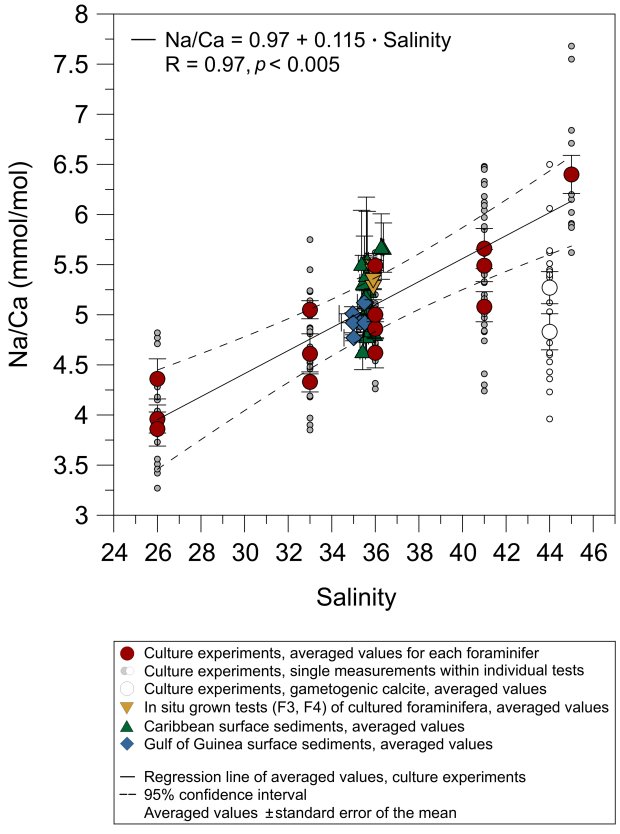

Figure 4. Na/Ca measurements of cultured *T. sacculifer* increase significantly with increasing salinity, while the temperature remained constant (26.5 °C ±0.25 °C). Each grey data point comprises averaged Na/Ca values of single map electron microprobe analysis along inner chamber wall cross sections of newly grown calcite (F, F-1) during the experiments. Related raw data of salinity experiments are given in Table 3. Red dots indicate averaged Na/Ca values of one foraminifer. Datasets of both temperature experiments (S 36, S 33) were added (Table 4, 5). The solid line marks the regression line of average values. Pearson's correlation coefficient R and its significance (*p*) is based on these averaged values. Dashed lines indicate the 95 % confidence interval. Vertical error bars are based on the standard error of the mean. Horizontal error bars demonstrate the salinity gradient between 0–60 m, reflecting the habitat range of *T. sacculifer*, expressed as standard error of the mean. Within each experiment Na/Ca values are normally distributed (Shapiro-Wilk test, Table A1). Data marked by yellow, blue and green symbols are not included into the calculation of the regression. Yellow reversed triangles indicate Na/Ca_{foram} values from in situ grown chambers (F-3, F-4) before culturing (annual salinity: ~35.9, temperature: ~27.4 °C, WOA13). Blue squares mark Na/Ca values of *T. sacculifer* from Gulf of Guinea surface sediments. Green triangles are data from Caribbean surface sediments (Table 2). White data points represent results of the 44-salinity experiment, in which cultured foraminifera underwent gametogenesis (Nürnberg et al., 1996).

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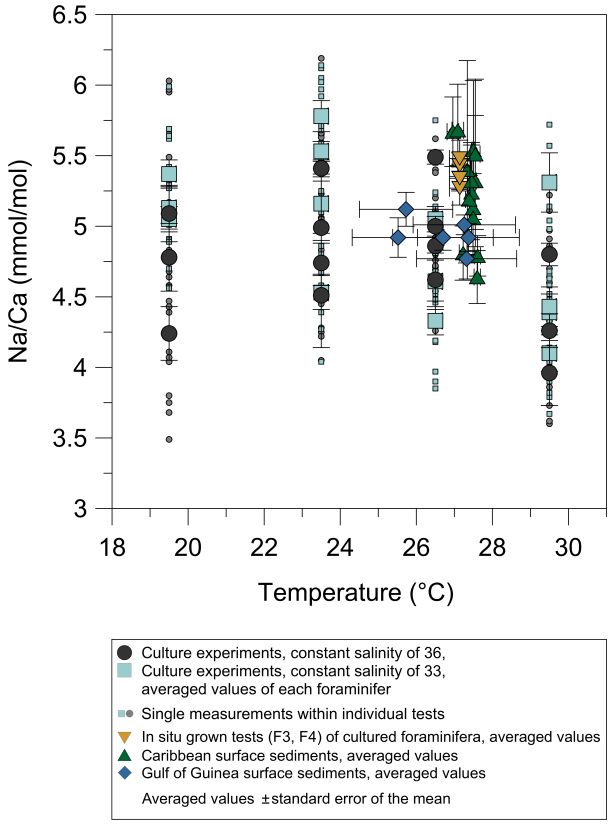

Figure 5. Na/Ca values of cultured *T. sacculifer* versus temperature show no significant correlation with increasing temperature (19.5 °–29.5 °C) in two different settings at either a constant salinity of 33 (S 33, p <0.14 – grey dots) or 36 (S 36, p <0.69 – light blue squares). Vertical error bars are based on the standard error of the mean. Each small light grey and light blue data point at every temperature experiment comprises Na/Ca values of single maps along chamber wall cross sections of newly grown calcite during culture experiments. Related raw data are given in Table 4 and 5. Yellow reverse triangles indicate Na/Ca values from in situ grown chambers before culturing (annual salinity: ~35.9, temperature: ~27.4 °C, WOA13). Blue diamonds reflect Na/Ca values of *T. sacculifer* from Gulf of Guinea surface sediments and green triangles are data from Caribbean surface sediments (Table 2). Horizontal error bars demonstrate the temperature gradient between 0-60 m, reflecting the habitat range of *T. sacculifer*, expressed as the standard error of the mean.

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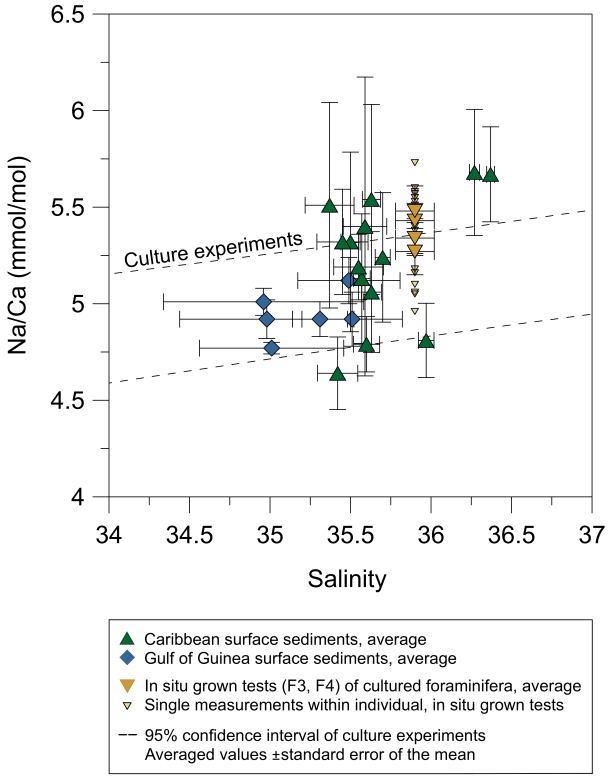

Figure 6. Na/Ca values of *T. sacculifer* from surface sediment samples collected in the Caribbean (stations SO164−01−3 to −50−3, green triangles) and the Gulf of Guinea (stations M6−5 GIK 16808−16869, blue diamonds) (Figure 1, Table 2). Dashed lines indicate the 95 % confidence interval derived from culture experiments (Figure 3) for comparison. Vertical error bars denote the standard error of the mean derived from replicate measurements (Table 2). Horizontal error bars visualize the salinity range from 0−60 m at the respective core locations (Figure 1), representing the possible habitat migration of *T. sacculifer* (Hemleben et al., 1987; Schmuker and Schiebel, 2002).

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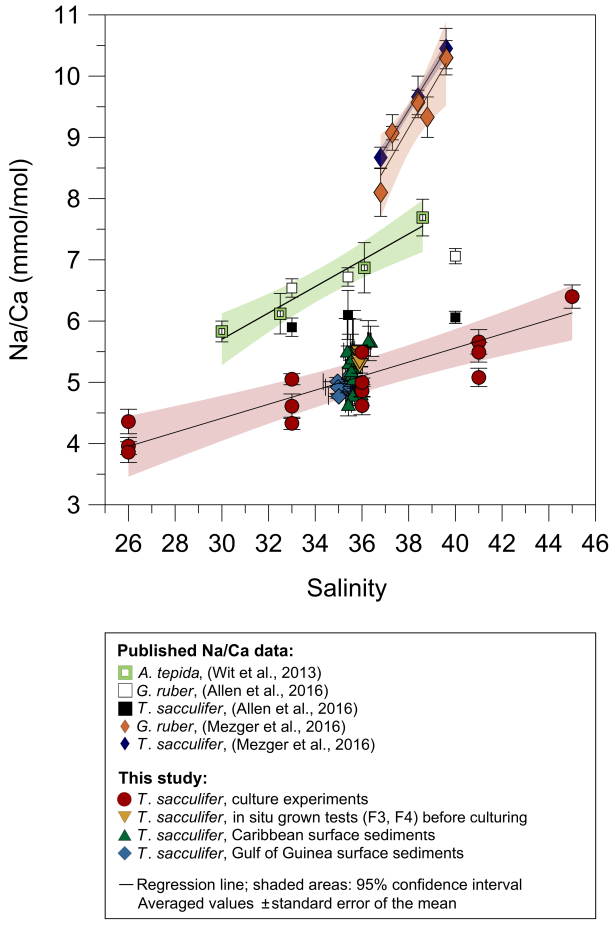

Figure 7. Comparison of this study's *T. sacculifer* Na/Ca values from culture experiments and surface sediments *versus* salinity with published Na/Ca data of benthic (Wit et al., 2013) and planktonic foraminifera (Allen et al., 2016; Mezger et al., 2016). All Na/Ca values are mean values from 25–40 specimens per analysis, except those of this study´s culture experiments. Here, each red and yellow symbol respresents averaged Na/Ca values of one foraminifer. Solid lines mark the regression of averaged values and shaded areas define the 95 % confidence interval for each study. Regression lines are based on the following equations. Wit et al., 2013: $Na/Ca_{A.\ tepida} = 0.22 \cdot S - 0.75$ ($R^2 = 0.96$, $p < 0.01$); Allen et al., 2016: $Na/Ca_{G.\ ruber\ (pink)} = 0.074\ (\pm 0.006) \cdot S + 4.1\ (\pm 0.2)$, ($R = 0.99$, $p < 0.01$), no significant relationship of Na/Ca with salinity in *T. sacculifer*; Mezger et al., 2016: $Na/Ca_{G.\ ruber\ (w)} = 0.57 \cdot S - 12.38$ ($R^2 = 0.91$, $p < 0.001$); $Na/Ca_{T.\ sacculifer} = 0.60 \cdot S - 13.49$ ($R^2 = 0.99$, $p < 0.001$); This study: $Na/Ca_{T.\ sacculifer} = 0.97 + 0.115 \cdot$ Salinity ($R = 0.97$, $p < 0.005$). Vertical error bars are based on the standard error of the mean and horizontal error bars demonstrate the salinity gradient between 0–60 m water depth, the possible habitat range of *T. sacculifer*.

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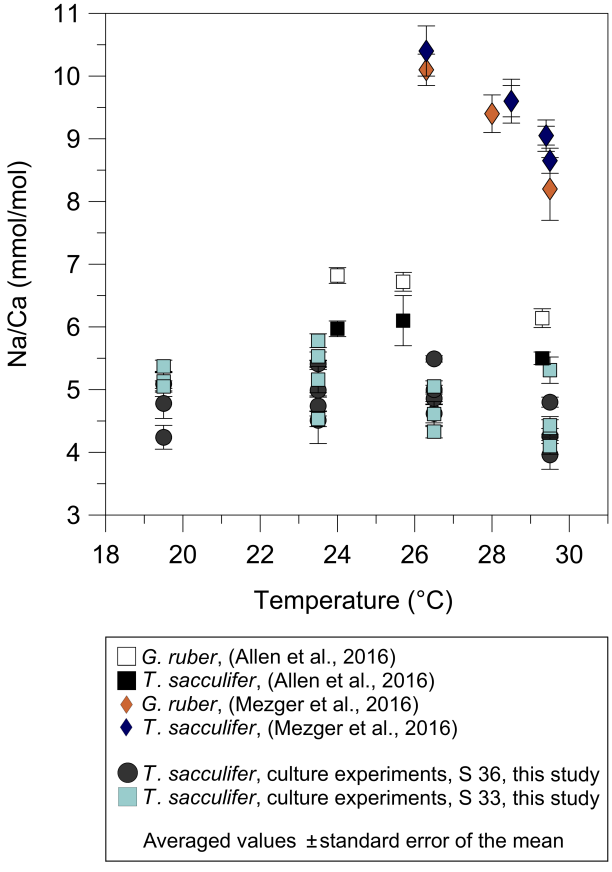

Figure 8. Comparison of this study´s *T. sacculifer* Na/Ca values from culture experiments versus temperature with published Na/Ca data of planktonic foraminifera from Allen et al. (2016) and Mezger et al. (2016). The salinity remained constant. Except for specimens from the Red Sea (*G. ruber*: $R^2 = 0.84$, $p < 0.001$; *T. sacculifer*: $R^2 = 0.95$, $p < 0.001$; Mezger et al., 2016), all foraminiferal Na/Ca data show no significant response to temperature changes. Vertical error bars are based on the standard error of the mean.

Appendices

Appendix A

Table A1. Na/Ca values generated for each experiment were tested for normality with the Shapiro-Wilk W-test and its corresponding *p*-value. If *p* is ≥ 0.05 (95 % confidence interval), the data are normally distributed. The number of measurements is indicated with n. Salinity and temperature (T) values derive from culture experiments listed in Table 3, 4 and 5.

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Salinity	T (°C)	n	W	*p*
26	26.5	20	0.962	0.587
41	26.5	34	0.953	0.590
44	26.5	24	0.972	0.709
45	26.5	12	0.878	0.083
33	19.5	26	0.960	0.399
33	23.5	31	0.952	0.183
33	26.5	29	0.971	0.590
33	29.5	29	0.941	0.108
36	19.5	27	0.964	0.462
36	23.5	24	0.960	0.440
36	26.5	26	0.975	0.767
36	29.5	24	0.951	0.286

Appendix B

Table B1. Inter- and intra-test Na/Ca variability of cultured *T. sacculifer* with varying experimental settings. The Na/Ca intra- and inter-test variability is calculated from single averaged maps (Table 3, 4, 5) within one foraminifer (#) and between foraminifera ($Na/Ca_{average}$ ±standard error of the mean) from the same experiment. The variability is expressed as the relative standard deviation (RSD) in %. Annual salinity and temperature data for in situ grown foraminiferal calcite (F-3, F-4) in the open ocean were taken from WOA13 (Zweng et al., 2013; Locarnini et al., 2013).

Sample #	**Intratest variabilty RSD (%)**	**Intertest variabilty RSD (%)**	Temp (°C)	Salinity	$Na/Ca_{average}$ (mmol mol^{-1})
7912	10.39	6.53	26.5	26	4.36 ±0.20
7913	10.26		26.5	26	3.96 ±0.14
7914	11.74		26.5	26	3.86 ±0.17
8135	13.18	5.53	26.5	41	5.66 ±0.20
8137	11.06		26.5	41	5.49 ±0.16
8138	7.43		26.5	41	5.08 ±0.15
7703	12.22	6.20	26.5	44	4.83 ±0.18
7704	10.73		26.5	44	5.27 ±0.16
8301	10.40	–	26.5	45	6.40 ±0.19
1.2	13.50	8.86	19.5	36	4.26 ± 0.58
1.3	12.38		19.5	36	4.78 ± 0.59
1.4	12.56		19.5	36	5.09 ± 0.64
2.1	7.51	7.79	23.5	36	4.74 ± 0.36
2.2	3.53		23.5	36	5.40 ± 0.18
2.3	16.21		23.5	36	4.99 ± 0.81

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2.4	8.13		23.5	36	4.51 ± 0.37
3.1	6.36	7.75	26.5	36	4.62 ± 0.29
3.2	5.32		26.5	36	4.65 ± 0.15
3.4	3.98		26.5	36	4.94 ± 0.32
3.5	1.83		26.5	36	5.77 ± 0.11
4.1	5.39	9.78	29.5	36	4.80 ± 0.26
4.3	12.70		29.5	36	3.96 ± 0.50
4.4	8.61		29.5	36	4.25 ± 0.35
5.1	4.76	3.19	19.5	33	5.37 ± 0.26
5.2	9.96		19.5	33	5.13 ± 0.51
5.5	5.26		19.5	33	5.05 ± 0.27
6.1	3.57	10.22	23.5	33	5.53 ± 0.20
6.2	6.72		23.5	33	4.52 ± 0.30
6.3	9.30		23.5	33	5.16 ± 0.48
6.5	6.30		23.5	33	5.75 ± 0.36
7.1	6.34	7.44	26.5	33	4.47 ± 0.43
7.3	4.88		26.5	33	5.05 ± 0.25
7.4	7.25		26.5	33	4.93 ± 0.21
7.5	10.56		26.5	33	4.60 ± 0.49
8.1	8.34	11.51	29.5	33	4.39 ± 0.37
8.3	9.25		29.5	33	4.10 ± 0.38
8.4	8.88		29.5	33	4.43 ± 0.39
8.5	7.79		29.5	33	5.31 ± 0.41
In situ grown chambers (F-3, F-4)					
4.1	5.63	1.69	27.4	35.9	5.27 ±0.12
2.3	3.25		27.4	35.9	5.43 ±0.18
7704	1.66		27.4	35.9	5.48 ±0.04
7703	3.85		27.4	35.9	5.34 ±0.08

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