Exploring foraminiferal Sr/Ca as a new carbonate system proxy

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abstract

In present day paleoclimate research one of the most pressing challenges is the reconstruction of atmospheric CO_2 concentrations. A variety of proxies for several components of the marine inorganic carbon system have been developed in this context (e.g. B isotopes, B/Ca, U/Ca) to allow reconstruction of past seawater pH, alkalinity etc., and thereby facilitate estimates of past atmospheric pCO_2. Based on culture experiments using the benthic foraminifera *Ammonia sp*. we describe a positive correlation between Sr/Ca and the carbonate system, namely DIC/ bicarbonate ion concentration. Foraminiferal Sr/Ca ratios provide potentially additional constraints on the carbonate system proxy, because the analysis of foraminiferal carbonate Sr/Ca is straightforward and not easily contaminated. Applying our calibration to a published dataset of paleo-Sr/Ca suggests the validity of Sr/Ca as a carbonate system proxy. Furthermore, we explore how our data can be used to advance conceptual understanding of the foraminiferal biomineralization mechanism.

1. Introduction

Incorporation of elements dissolved in seawater (e.g. Na, Mg, Sr, B) into biogenic calcium carbonate during calcification is influenced by a combination of various environmental parameters and biological processes. Element to calcium ratios in the calcium carbonate of calcifying organisms, such as foraminifera and corals, are therefore used to reconstruct past oceanographic conditions (Boyle, 1988; Delaney et al., 1985; Emiliani, 1955). The Mg/Ca ratio of foraminiferal tests, for instance, is a relatively established reconstruction tool for past seawater temperatures (Nürnberg et al., 1996). Due to ongoing anthropogenic CO_2 emissions and concomitant acidification of sea surface waters (i.e. ocean acidification) studying past changes in seawater carbonate chemistry has become a major focus in paleoclimate research (e.g. Hönisch et al., 2012; Bijma et al., 2013). Particularly rapid acidification events in the geological past such as associated with the PETM (Paleocene–Eocene Thermal Maximum) might provide important constraints for forecasting shifts in ecosystem and or community composition during such transitions (Kiessling and Simpson, 2011; Rodríguez-Tovar et al., 2011). Insight into (long-term) marine carbon cycling and perturbations therein critically depend on robust reconstruction of at least two parameters of the seawater carbonate system (Zeebe and Wolf-Gladrow, 2001).

For reconstructing past seawater carbonate chemistry, foraminiferal test boron content and their stable isotope ratios are, for example, used (Hemming and Hanson, 1992; Hönisch et al., 2009; Sanyal et al., 1995,1999, 2000, 2001; Yu et al., 2007, 2010). Still, accurate determination of B/Ca and $\delta^{11}B$ remains challenging and the uptake of boron and its consequent incorporation and or speciation are not fully understood on a process level (Kaczmarek et al., 2015). Other C-system (carbonate system) proxies include for instance the Cd/Ca and Zn/Ca ratios in benthic foraminifera, since they correlate with the relative level of oversaturation (ΔCO_3^{2-}; Marchitto et al., 2000; 2002). Planktonic (Bijma et al., 2002; Broecker and Clark, 2002) and benthic (Keul et al., 2013a) foraminiferal test weight normalized to size has been shown to correlate with seawater carbonate ion concentration. Furthermore, it has been shown that foraminiferal U/Ca variability reflects changes in carbon speciation (Keul et al., 2013b; Raitzsch et al., 2011; Russell et al., 2004).

Of the different elements that are analyzed in carbonate, Sr is analytically one of the most robust, with high accuracy and precision. Values for Sr/Ca are hence relatively well-studied and have been related to different processes in a variety of organisms, such as temperature in corals (Smith and Roth, 1979), calcification- and growth-rates in coccolithophorids (Stoll and Schrag, 2000, 2001) and has been shown to be influenced by growth rates, temperature, salinity and pH in foraminifera (Kisakürek et al., 2008; Lea et al., 1999). Foraminiferal Sr/Ca has been shown to vary with water depth (e.g. McCorkle et al., 1995; Elderfield et al.; 1996, Lear et al., 2003; Rosenthal et al., 2006; Yu et al. 2014), which could be caused by parameters changing with depth, such as pressure, temperature (e.g. Rosenthal et al., 1997) and/ or carbonate ion concentration. It has been shown in many studies that foraminiferal Sr/Ca varies with seawater carbonate chemistry (e.g. Dissard et al., 2010; Dueñas-Bohórquez et al., 2009, 2011; Lea et al., 1999; Raitzsch et al., 2010; Russell et al., 2004). The effect seems to be species specific, because three planktonic species (*Globigerinoides sacculifer*, *Globigerina bulloides*, *Orbulina universa*) show an increase in Sr/Ca with increasing pH, whereas no response was observed for a benthic species (*Heterostegina depressa*) and another planktonic species (*Globigerinoides ruber*). For the benthic species *Ammonia sp.*, the response of Sr-incorporation to changing carbonate chemistry is ambiguous: while Dueñas-Bohórquez et al. (2009) observed no impact of changing carbonate chemistry, Dissard and colleagues (2010) found a distinct increase in Sr incorporation with increasing pH. Hence, whereas Sr/Ca is obviously sensitive to environmental parameters, response to these parameters is not univocal.

Compared to reconstructing temperature (based on e.g. foraminiferal Mg/Ca and $\delta^{18}O$), proxies for C-system parameters are generally less robust and reconstructions based thereon are thus susceptible to a number of uncertainties (Pagani et al., 2005). The source of uncertainty of C-system proxies stems, to a considerable extent, from the fact that the seawater C-system consists of six inter-correlated parameters (Zeebe and Wolf-Gladrow, 2001). This inter-correlation usually renders the identification of a single parameter causing changes to, e.g., B/Ca impossible. The only way to resolve this inter-relation is by experimentally deconvolving the impact of different C-system parameters on foraminiferal carbonate chemistry proxies (e.g. Kaczmarek et al., 2015, Keul et al., 2013b). Almost all experimental studies dealing with the C-system employed either a TA (total alkalinity) or a DIC (dissolved inorganic carbon) manipulation (also termed classical C-system manipulations). In both cases changes in, e.g., pH and carbonate ion concentration are

correlated, so that an observed effect cannot be traced back to an individual parameter such as pH or carbonate ion concentration. The latter information is crucial for a process based understanding of the potential impact of carbonate chemistry on Sr/Ca. In this context, especially the uptake mechanism of divalent ions is of interest. Several conceptual biomineralization models have been proposed for foraminifera (see de Nooijer et al., 2014b for an overview). It has long been recognized that foraminifera exert a tight control on their calcification process (Röttger, 1973; Spindler and Röttger, 1973; Hemleben et al., 1989). This is accomplished by precipitating calcite in a confined space (the so-called site of calcification, SOC or delimited biomineralization space, DBS). The DBS represents no significant ion-reservoir for chamber formation and therefore requires constant supply of Ca and DIC, which was originally assumed to be delivered by vesicles (Bentov and Erez, 2006; Erez, 2003). The exclusive transport of Ca by vesicles, however, makes it difficult to account for element partitioning. Therefore, an alternative ion transport model, although including vesicle transport, centrally features transmembrane ion transport (Glas et al., 2012; Keul et al., 2013b; Mewes et al., 2014; Nehrke et al., 2013). During trans-membrane Ca uptake, Sr is accidentally taken up because calcium transport proteins also admit other ions to a certain extent, especially Sr and Ba (Berman and King, 1990). Since transmembrane transport is tightly regulated by all eukaryotic cells, such an ion transport model explains the fine-tuned control on all relevant parameters concerning calcite precipitation.

In this study we use a non-classical, i.e. combined TA/DIC, manipulation of the C-system in order to identify parameters of the C-system affecting test Sr/Ca in the benthic foraminifer *Ammonia sp.* ("Ammonia molecular type T6", Hayward et al., 2004). The Sr/Ca-C-system relationship is subsequently applied to a published dataset of paleo-Sr/Ca to explore the validity of Sr/Ca as a C-system proxy in different species. We also present a calcification model which demonstrates that the effect of seawater carbonate chemistry on Sr partitioning is in line with current understanding of minor element partitioning in foraminifera.

2. Material & Methods

2.1. Sample Collection and Culturing

Surface sediments of intertidal mudflats of the Wadden Sea near Dorum, Germany, were sampled for foraminifera between January and March 2011. The macrofauna was removed upon return at the laboratory by sieving over a 1 mm-screen. Sieved sediments were transferred to aquariums, submerged in 0.2 μm filtered North Sea water (S=33, pH=8.1) and stored at 10°C. Prior to the culture experiments, living adult specimens of *Ammonia sp.* were isolated from these sediments and transferred to well plates at 25°C and fed every second day *Dunaliella salina*. After about one week ca. 10% of the adult specimen had reproduced asexually, yielding between 50 and 200 juveniles per reproduction event (De Nooijer et al., 2014a). After 2-3 days, these juveniles had built 3-4 chambers and were transferred into the culture experiments.

2.2. Seawater preparations

A total of eight culture media were prepared using sterile-filtered (0.2 μm pore size) North Sea water (see Keul et al., 2013b for details). Borosilicate flasks containing the manipulated seawater were sealed headspace-free and gas-tight with Teflon-lined caps and kept at 3°C until use in the experiments. The eight treatments were divided into two groups using two different C-system perturbations (Tab. 1):

a) "TA manipulation": four treatments, covering a range of [CO_3^{2-}] and pHs, while DIC remained constant.

b) "pH-stable manipulation": four seawater manipulations, where DIC and TA were manipulated as to keep pH constant, the corresponding pCO_2 values were matched to those of the TA-manipulation.

2.3. Experimental Setup and Culturing

The duration of the culture experiments was between 59 and 96 days, requiring an experimental setup that allowed maintaining the C-system parameters constant for this period. The experimental setup (see Keul et al., 2013b for details) consisted of four gas-tight boxes, through which air with four different CO_2 concentrations (Tab. 1) was purged. Juvenile foraminifera were placed into Petri dishes containing manipulated seawater, long-term stability was ensured by matching the CO_2 (aq) of the manipulated culture media to the pCO_2 in the gas-tight boxes. The complete setup was placed in a temperature-controlled room (26°C) with a natural day to night light cycle (12h/12h). Water in the Petri dishes was exchanged every two to three days. To ensure pre-equilibration of the borosilicate bottles containing the manipulated seawater, they were unsealed and stored in the boxes prior to water-exchange. After water exchange, foraminifera were fed heat-sterilized algae (*D. salina*). Petri dishes were exchanged every two weeks to minimize bacterial growth on the bottom of the dishes due to accumulation of waste material. Foraminifera were harvested at the end of the culture period, the organics removed with NaOCl, rinsed thoroughly with ultrapure water and dried.

2.4. Sample analysis

2.4.1. Carbonate chemistry

Acid-washed 13mL borosilicate flasks were filled headspace-free and kept at 0°C until measurement of DIC (within 3 days). DIC was measured photometrically in duplicates with a QuaAAtro autoanalyzer (Seal Analytical, Meqon, USA). Repeated measurements of a batch of North Sea seawater sampled in surface waters off Helgoland (Germany) were carried out to determine the average precision, which was ±10 µmol/kg-sw. Batches of seawater sampled in the same location (off Helgoland) were used earlier to determine the precision of the DIC measurements (e.g. Langer et al. 2009). Batch No. 54 of A. Dicksons Certified Reference Material Seawater (Marine Physical Laboratory, Scripps Institution of Oceanography) was measured to account for inaccuracies in the measurements. The pH was measured potentiometrically using a two-point NBS-calibrated glass electrode (Schott Instruments, Mainz, Germany) interfaced to a WTW pH-meter. Conversion to total scale was achieved by the simultaneous measurements of a Tris seawater buffer (Tris/Tris-HCl buffer in artificial seawater, Dickson et al., 2007) and values reported here are on this scale. A conductivity meter (WTW Multi 340i) interfaced to a TetraCon 325 sensor was used to measure temperature and salinity. Input parameters DIC and pH (Hoppe et al., 2012) were used to calculate the C-system parameters using the CO2SYS program adapted to Excel by Pierrot et al., 2006. The equilibrium constants K1 and K2 were used of Mehrbach et al. (1973), as reformulated by Dickson and Millero (1987).

2.4.2. Elemental concentration of culturing media

Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to determine the strontium and calcium concentration of the eight manipulated seawater treatments. Calcium (Ca^{2+}) was measured at a wavelength of 316 nm, Strontium (Sr^{2+}) at 408 nm (Table 1), with a relative standard deviation <1%.

2.4.3. Elemental composition of foraminiferal calcite

The LA-ICP-MS (laser ablation-inductively coupled plasma-mass spectrometry) system at Utrecht University (Reichart et al., 2003) was used to determine elemental concentrations of cleaned foraminiferal tests. It consists of a sector-field mass spectrometer (Element 2, Thermo Scientific) connected to an excimer laser (Lambda Physik), equipped with GeoLas 200Q optics. The pulse repetition rate was set to 6 Hz, energy density was ~1 J cm^{-2} and ablation beam diameter was set at 80 μm. Counts of ^{24}Mg, ^{27}Al, ^{43}Ca ^{44}Ca, ^{55}Mn, ^{88}Sr, ^{238}U were used to calculate elemental concentrations in the samples. A complete measurement cycle through all masses took 0.52 s. The ablation profiles were checked for potential surface contamination using ^{27}Al. A NIST glass (SRM NIST 610) was ablated three times and an in-house matrix-matched calcite once between every 10 samples. Relative variability for this calcites Sr/Ca during measurements was <3 % (rsd), matching variability reported in Raitzsch et al. (2010). The NIST610 was ablated at a higher energy density (~5 J cm^{-2}). Assuming 40 weight % calcium in calcite, ^{43}Ca was used as an internal standard, while counts for ^{44}Ca were used to check for consistency. Using the software package Glitter (software for data reduction, GEMOC; Van Achterbergh et al., 2001, Griffin et al., 2008), time resolved counts during ablation of the test were integrated and the background subtracted. Isotopic counts were standardized to ^{43}Ca and converted to concentrations using the signals obtained on the NIST glass assuming standard natural isotopic abundances and values reported by Jochum et al. (2011).

Six individual specimens (seven in treatment A1) were analyzed per treatment and between five to seven individual laser spot measurements were obtained for each specimen, together equaling 276 single spot measurements. During the measurements, the laser protruded sequentially deeper parts of the test and measurements were stopped once the laser had protruded through the test. Five ablation profiles were discarded because the chamber walls were too thin and the resulting ablation profiles too short for reliable determination of element/Ca ratios. Using measured seawater Sr/Ca ratios, the empirical partition coefficient for strontium in foraminiferal calcite (D_{Sr}) was calculated according to:

$$D_{Sr} = \frac{(\frac{Sr}{Ca})_{CC}}{(\frac{Sr}{Ca})_{SW}} \quad (1)$$

where $(Sr/Ca)_{CC}$ refers to the measured molar ratio of Sr and Ca in foraminiferal calcite and $(Sr/Ca)_{SW}$ to that in the culture media.

Variability in the foraminiferal Sr/Ca, as expressed by the relative standard deviation (rsd, defined as the ratio of the standard deviation ('sigma') to the mean, expressed in percent), was with 7.2-15.3% relatively low for LA-ICP-MS measurements on foraminifera, when compared to ICP-MS analyses of whole, acid-dissolved tests, where intratest inhomogeneity cannot be resolved. There was no detectable effect of the treatments on variability in obtained Sr/Ca. Average intratest variability (9.2% rsd) was lower than average intertest variability (15.3% rsd). Uncertainty in the measurements can arise from measuring too few single spots/

individuals. This can be expressed as standard error (defined as the ratio of the standard deviation to the square root of the sample size, here number of measurements) and is calculated from the rsd, with lower errors associated with increasing number of measurements. When measuring 5, 10, or 20 single spots per individual, the standard errors are 4, 3 and 2% respectively and when measuring 5, 10, or 20 individuals, the associated uncertainty is 3, 5 and 7%, respectively. Consequently, the 5-7 measurements per individual test entail an uncertainty of 3.5-4.1%, and the limitation to 6-7 individuals entails a standard error of 6.2-5.8%.

2.4.4. Foraminiferal growth

Foraminiferal growth rates were determined from the final weight of dead specimens (Mettler Toledo UMX 2 Ultra Microbalance, 0.1 μg precision), as described in (Keul et al., 2013a). Growth rates are defined as the mass of calcite grown per specimen per unit time (μg/d/ind). It should be noted, that this refers to bulk growth rate. Instantaneous growth rate (e.g. during the intermittent addition of new chambers) cannot be assessed this way.

2.5. Statistics

The statistics environment R (R Development Core Team, 2012; http://www.R-project.org) was used for statistical analysis. Analysis of variance (ANOVA) was performed to determine the effect of the individual parameters of the C-system on foraminiferal Sr/Ca. Log transformation ensured normally distributed data (Shapiro test, $p > 0.05$) and the null-hypothesis of the ANOVA (all group means are equal) was rejected at the 5% level ($p < 0.05$). Average values and 2SE (standard errors) are reported throughout the text.

3. Results

3.1 Foraminiferal Sr/Ca and D_{sr}

Average foraminiferal Sr/Ca values range from 1.1 to 1.7 mmol/mol, with individual spot measurements ranging from 0.85 to 2.20 mmol/mol. The range in the distribution coefficient for strontium, D_{Sr} (the ratio of Sr/Ca_{CC} to Sr/Ca_{SW}), has a similar magnitude in individual measurements and means: 0.13 to 0.37 for individual spot measurements, while mean D_{Sr} ranges from 0.17 to 0.29. The highest strontium concentration was found in tests of individuals cultured in treatment B4, characterized by the highest pCO_2 and highest DIC of the pH-stable treatment. The Sr/Ca and consequently D_{sr} are relatively homogeneous in tests grown in the TA manipulation (A1-A4), whereas the range in Sr/Ca and D_{Sr} from individuals grown in the pH-stable manipulation is more variable (B1-B4, Fig. 1).

3.2. Correlation between foraminiferal Sr/Ca and C-system

Single spot measurements from the same specimen were averaged and linear regression analyses were performed to analyze correlation between individual C-system parameters and foraminiferal Sr/Ca. Results of the regression analyses (intercept and slope) and the statistical output (p, R^2 and F) are summarized in Tab. 2. All regressions were highly significant ($p < 0.05$) and explain, depending on the C-system parameter, between 23 and 64% of the observed variability in D_{Sr}. The corresponding plots of the regression analyses are shown in Fig. 1.

4. Discussion

4.1. Sr incorporation in other foraminiferal species

Previous culturing studies on *Ammonia sp.* reported D_{Sr} values of 0.15-0.18 (Dissard et al., 2010), 0.16-0.17 (Raitzsch et al., 2010), 0.16-0.19 (Dueñas-Bohórquez et al., 2011), 0.16-0.20 (De Nooijer et al., 2014a), which is in good agreement with the range of mean D_{Sr} of 0.19-0.20 determined on specimens from the TA-manipulation (Fig. 2, blue crosses). The D_{Sr} values of the tests grown in the pH-stable manipulation (Fig. 2; 0.17-0.29) are higher and are higher and indicate that the C-system parameters primarily affecting foram D_{Sr} must be changing D_{Sr}, whereas it must be relatively constant in the other manipulation (see below). The D_{Sr} varies among foraminiferal taxa, often resulting in higher values than that of *Ammonia sp.* (e.g. *M. vertebralis* 0.17, *A. lessonii* 0.29, *A. hemprichii* 0.32, *H. depressa* 0.27-0.33, *N. calcar* 0.35, values taken from (Dueñas-Bohórquez et al., 2011; Raitzsch et al., 2010; Raja et al., 2005). Despite this variability in D_{Sr}, all foraminiferal values are higher than inorganic D_{Sr} values in the absence of Mg (ranging from 0.02 to 0.14; Lorens, 1981; Nehrke et al., 2007; Tesoriero and Pankow, 1996).

Field studies showed that partition coefficients (D_{Sr}) range between 0.1-0.2 for *Uvigerina* spp., *Cibicidoides* spp., *Melonis barleeanum* and *Hoeglundina elegans* collected from core tops at various ocean basins (McCorkle et al., 1995; Elderfield et al., 1996; Rosenthal et al., 1997; Reichart et al., 2003; Lear et al., 2003; Yu et al., 2014). These studies noted an impact of pressure (Elderfield et al., 1996; Rosenthal et al., 1997) and preferential dissolution (McCorkle et al., 1995) on benthic foraminiferal Sr/Ca. In the field, temperature, salinity, pH and $[CO_3^{2-}]$ do not have a noticeable impact on Sr/Ca (Yu et al., 2014) and the effect of pressure and post-mortem dissolution may be explained by the impact of $\Delta[CO_3^{2-}]$ on Sr incorporation (Yu et al., 2014). These core-top series, however, do not allow to disentangle

impact of [DIC] versus $\Delta[CO_3^{2-}]$ on foraminiferal Sr/Ca values since with increasing water depth, [DIC] generally increases, while saturation state decreases (Edmond and Gieskes, 1970). In the field, Sr/Ca decreases with lower $\Delta[CO_3^{2-}]$ (more corrosive conditions), which is parallel to an increase (our results) due to a higher [DIC]. These opposing effects of DIC and $\Delta[CO_3^{2-}]$ on foraminiferal Sr/Ca may dampen the so far reported effect of $\Delta[CO_3^{2-}]$ on Sr incorporation from environmental samples.

4.2. Correlation between foraminiferal Sr/Ca and C-system

Two different manipulations were used to alter seawater carbonate chemistry: TA manipulation (A1-A4) and pH-stable manipulation (B1-B4), while maintaining similar Sr/Ca_{sw} across treatments (Table 1). The C-system parameters co-vary in both sets, but in a different way. As a result, certain parameters can be excluded as primary controlsw on observed changes in foraminiferal Sr/Ca. We can assume that if a certain C-system parameter is controlling incorporation of Sr into foraminiferal calcite, the correlation (i.e. trend or slope) between Sr/Ca and this parameter should be similar in both manipulations. Since this is not the case for pCO_2 and carbonate ion concentration (Fig. 1c), these parameters do not exert the main controls on Sr/Ca in the species studied here.

In the case of pH, Sr/Ca values of the pH stable treatment exhibit a larger variability (Δ in Sr/Ca of 0.8) when compared to the TA-manipulation treatment (Δ is 0.2). If pH would have controlled Sr/Ca, the opposite should be true (constant Sr/Ca in the pH stable treatment and full range of variation in the TA manipulation). Since this is not the case (Fig. 1f), pH does not affect Sr-incorporation. This is also reflected in the results of the regression model (Table 2): while p (the significance level of the test's outcome) and F (the ratio between the sample means variances and the variation within samples) remain statistically significant ($p<0.05$, $F=12.43$), the R^2 value is small (0.04) indicating that there is no predictive power of pH on foraminiferal Sr/Ca, as also indicated by the confidence intervals (Fig. S1). The conclusion that carbonate ion concentration and pH do not affect Sr/Ca in *Ammonia* is in accordance with one earlier study (Dueñas-Bohórquez et al., 2011), but in conflict with another (Dissard et al., 2010). This discrepancy might be explained by the experimental setup employed by Dissard et al. (2010), who changed salinity and temperature in addition to carbonate chemistry. Synergistic effects on calcification of, e.g., temperature and carbonate chemistry were reported for coccolithophores (Sett et al., 2014). Such synergistic effects might also be present in foraminifera, highlighting the importance of the experimental setup when comparing results from different studies. The observation that either carbonate ion concentration, CO_2, or pH affect Sr/Ca in e.g. *O. universa* (Russell et al., 2004) emphasizes the need to consider species-specific effects as has been shown for coccolithophores (Langer and Bode, 2011; Langer et al., 2006a).

Regarding the regression between Sr/Ca and bicarbonate ion concentration (Fig. 1b), it should be noted that the range in bicarbonate ion concentration is relatively small in the TA-manipulation (approximately 250 μmol/kg-sw), likely explaining the reduced significance of the regression ($p= 0.02$) when compared to the pH-stable manipulation ($p< 2e-16$). When taken at face value, the correlation of Sr/Ca and bicarbonate ion concentration in the TA-manipulation excludes bicarbonate ion concentration as a control on Sr/Ca. However, the small range in bicarbonate ion concentration in the TA-manipulation most likely renders this correlation meaningless. This conclusion is supported by the fact that all bicarbonate ion concentration values of the TA-manipulation fall on the regression line of the pH-stable-

manipulation (Fig 1b). Therefore, bicarbonate ion concentration might control Sr/Ca. A similar argument applies to TA and DIC (Fig 1d, e). Please note that bicarbonate ion concentration is the major constituent of both TA and DIC (Tab. 1). Therefore, bicarbonate ion concentration might be instrumental in the Sr/Ca change, whereas TA and DIC might merely accidentally correlate with Sr/Ca. This possibility is explored in detail in the next section.

4.3. Biomineralization insights

Why should Sr/Ca in *Ammonia* be influenced by bicarbonate ion concentration, TA and/ or DIC? Since the correlations between TA and DIC and Sr/Ca is positive, it might be hypothesized that growth rate is the mediating agent (see also Russell et al., 2004; Dissard et al., 2010). This is, however, unlikely since 1) carbonate ion concentration influences growth rate in *Ammonia* more than the other C-system parameters (Keul et al., 2013a) and 2) in the present data set no correlation was observed between foraminiferal Sr/Ca and growth rate ($p > 0.05$). One of the central parameters in this context is the super-saturation of the DBS fluid with respect to calcite, the so called omega (Fig. 3). The DBS omega is the main control on calcite precipitation rate (Nielsen, 1964). The latter is probably regulated and fine-tuned by the organism in order to exert the necessary control on morphogenesis, chamber- and whole-test-growth rate. The DBS omega will be determined by both the influx of Ca and of DIC. Please note that DBS pH is kept high during chamber formation, facilitating the conversion of CO_2 to bicarbonate and eventually carbonate ion (De Nooijer et al., 2009a; 2009b; 2014b Glas et al., 2012). In our experiment, we increased the seawater DIC/bicarbonate concentration beyond typical sea surface water levels, thereby substantially increasing the flux of DIC into the DBS. This flux might be mediated by both bicarbonate ion concentration via membrane transporters, and CO_2 via diffusion through the lipid bilayer (Fig. 4). At least the diffusion of CO_2 cannot be controlled by the organism due to the steep pH gradient between DBS and microenvironment (De Nooijer et al., 2009a; Bentov et al., 2009; Glas et al., 2012). This enhanced DIC influx may elevate the omega ($\Omega_{calcite}$) at the DBS. We assume that foraminifera counteract this rise in omega by reducing the DBS Ca influx. This assumption is reasonable, because all eukaryotic cells have a sophisticated machinery to regulate Ca fluxes (Medvedev, 2005). Reduced Ca influx will affect the Sr/Ca ratio of the DBS and therewith the D_{Sr} in relation to DIC (Fig. 4).

4.4. Mathematical Exploration

The transmembrane transport model can be applied to the transport pathway of divalent cations (e.g. Mg, Sr and Ca) to describe the trace element to Ca ratios in the tests of foraminifera as a function of the trace element to Ca ratio in seawater. Here we explore the impact of DIC on foraminiferal Sr/Ca. A significant fraction of the carbon needed for calcification is probably due to passive diffusion of CO_2 into the cell (the "cheapest" option). In addition to that, HCO_3^- probably needs to be actively transported into the cell to sustain high calcification rates. Both CO_2 and HCO_3^- enter the DBS, where they are converted to CO_3^{2-}, due to the high pH inside the DBS (see Fig. 4). The transport pathway of DIC species is not included in the recently proposed transmembrane transport model (Nehrke et al., 2013), but due to oversaturation considerations also of importance for minor element partitioning. Here, we fill this gap by assuming a tight regulation of calcification rate by foraminifera which may explain the linear dependence of D_{Sr} on DIC.

The value of D_{Sr} can be calculated according equation (11) in Langer et al. 2006b.
In order to describe different ionic compositions in seawater and in the DBS fluid we replace the concentrations by activities. The activity of Ca^{2+} in a compartment x, denoted by $\{Ca^{2+}\}_x$, is related the Ca^{2+} concentration $[Ca^{2+}]_x$ by the activity coefficient γ_x: $\{Ca^{2+}\}_x = \gamma_x [Ca^{2+}]_x$. The activity coefficient for Ca^{2+} in seawater, γ_{SW}, is around 0.2. In the DBS, the activity coefficient γ_{DBS} is assumed to be regulated by the cell, and probably range from 0.2 to almost 1.0. In terms of activities equation (11) in Langer et al. 2006b is given by:

$$D_{Sr} = D_{Sr}^0 \frac{\{Ca^{2+}\}_{SW}}{\{Ca^{2+}\}_{DBS}} = D_{Sr}^0 \frac{\gamma_{SW}}{\gamma_{DBS}} \frac{[Ca^{2+}]_{SW}}{[Ca^{2+}]_{DBS}}, \tag{2}$$

where D^0_{Sr} is the equilibrium value of D_{Sr}, $[Ca^{2+}]_{SW}$ the calcium concentration in seawater, and $[Ca^{2+}]_{DBS}$ the calcium concentration at the site of calcification. $[Ca^{2+}]_{DBS}$ and the internal carbonate ion concentration $[CO_3^{2-}]_{DBS}$ determine the level of supersaturation of the DBS fluid with respect to calcite:

$$\Omega = \frac{\{Ca^{2+}\}_{DBS}\{CO_3^{2-}\}_{DBS}}{K_S} = \frac{[Ca^{2+}]_{DBS}[CO_3^{2-}]_{DBS}}{K^*_{Sp}}, \tag{3}$$

with the thermodynamic solubility constant, K_S, and the so-called stoichiometric seawater saturation product K^*_{Sp}. It is assumed that $\Omega_{calcite}$ is actively controlled by the foraminifera (homeostasis) to regulate calcification rate during chamber formation.
As there is no evidence for the existence of CO_3^{2-} ion channels or pumps and the equilibration between CO_3^{2-} and HCO_3^- is almost instantaneous, $[CO_3^{2-}]_{DBS}$ can be described as a constant fraction f of the bicarbonate ion concentration in the DBS ($[HCO_3^-]_{DBS}$; for a given pH): $[CO_3^{2-}]_{DBS} = f[HCO_3^-]_{DBS}$. For an internal pH of 9 (Bentov et al., 2009, de Nooijer et al. 2009) the value of f is 1.14. Then, we obtain from equations (2) and (3) an expression of D_{Sr} in terms of $\Omega_{calcite}$ and $[HCO_3^-]_{DBS}$:

$$D_{Sr} = D_{Sr}^0 \frac{\gamma_{SW}}{\gamma_{DBS}} \frac{[Ca^{2+}]_{SW} f}{\Omega K^*_{Sp}} [HCO_3^-]_{DBS}. \tag{4}$$

The value of $[HCO_3^-]_{DBS}$ can be estimated using a simple balance equation for the flux of bicarbonate in/out of the DBS. For simplicity, we assume a constant influx of bicarbonate, HC_{inf}, which results from the conversion of CO_2 (diffusing in passively) to HCO_3^- and, depending on the rate of calcite precipitation, also an active HCO_3^- transport. We further assume that foraminifera regulate both $[CO_3^{2-}]_{DBS}$ and $[Ca^{2+}]_{DBS}$ to control $\Omega_{calcite}$ and thus the rate of calcite precipitation, $\sim(\Omega_{calcite} - 1)^n$, at a favorable level. For this $\Omega_{calcite}$ -homeostasis bicarbonate influx and/or efflux via ion channels (with a rate $\sim\Delta[HCO_3^-]$) may play a key role in controlling $[HCO_3^-]_{DBS}$ and therewith $[CO_3^{2-}]_{DBS}$. Within this model $[HCO_3^-]_{DBS}$ is described in terms of the simple differential equation

$$\frac{d[HCO_3^-]_{DBS}}{dt} = k_1\left([HCO_3^-]_{SW} - [HCO_3^-]_{DBS}\right) - k_2(\Omega - 1)^n + HC_{inf}, \tag{5}$$

which for the assumption that Ω = constant has the steady state solution

$$\left[HCO_3^-\right]_{DBS} = \left[HCO_3^-\right]_{SW} + \frac{HC_{\inf} - k_2(\Omega - 1)^n}{k_1}. \qquad (6)$$

We describe the concentration of bicarbonate in the seawater as a constant fraction *g* of dissolved inorganic carbon, i.e. $[HCO_3^-]_{SW} = g[DIC]$. When all experimental carbonate chemistry data are plotted in DIC – $[HCO_3^-]$ space, a linear regression with a slope of 0.895 can be fitted.

Then, we obtain from the equations (4) and (6) the following linear equation for the DIC dependence of D_{Sr}:

$$D_{Sr} = m \cdot [DIC] + n, \qquad (7)$$

with the slope

$$m = D_{Sr}^0 \frac{\gamma_{SW}}{\gamma_{DBS}} \frac{[Ca^{2+}]_{SW} fg}{\Omega K_{Sp}^*} \qquad (8)$$

and the intercept

$$n = D_{Sr}^0 \frac{\gamma_{SW}}{\gamma_{DBS}} \frac{[Ca^{2+}]_{SW} f}{\Omega K_{Sp}^*} \left(\frac{HC_{\inf} - k_2(\Omega - 1)^n}{k_1} \right). \qquad (9)$$

We use the experimental slope m = 2* 10^{-5} μmol^{-1} l to calculate Ω from the internal pH. Assuming a pH of 9 at the site of calcification (DBS) and solving equation 7 for Ω, yields an Ω range of 5.4 - 26.9 (when γ_{SW}/γ_{DBS} = 0.2 -1.0, S = 33, T = 25, D^0_{Sr} = 0.021, $K^*_{Sp} = 10^{-6.4}$ $mol^2 kg^{-2}$ and $[Ca^{2+}]_{SW}$ = 10 mmol l^{-1}). This relative high Ω is comparable to *in vivo* levels of Ω occurring during shell formation of an oyster. For this organism the Ω value in the extrapallial fluid (the medium of shell formation) was estimated to be in the range of about 16 to 27 (Sikes et al., 2000), indicating the feasibility of our model.

We want to stress, that the here proposed model is a mathematical exploration of the observed dependency of D_{Sr} on seawater carbonate chemistry. In this model, the parameter of the carbonate system, which is instrumental in altering D_{Sr} is DIC. Based on our experimental results we identified either bicarbonate ion concentration, DIC or TA as the parameter affecting D_{Sr}. Taken together, the experimental results and the model lead us to conclude that bicarbonate ion concentration, DIC is the driving force behind the D_{Sr} change. This conclusion is strongly supported when considering that TA cannot cause any changes in cell physiology. The reason is that TA is a conceptual parameter, which is of great importance in determining the C-system (Dickson, 1992) but has no "physical reality", which could be experienced by an organism.

The model proposed here is a continuation of a recent model for foraminiferal calcification (Nehrke et al., 2013). This model is based on the transmembrane transport of Ca^{2+} from seawater into the DBS and accidental uptake of other divalent cations and subsequent incorporation in the calcite crystal lattice. One way to test the model would be to measure other divalent cations, which are subjected to the same transport mechanism and should therefore display the same relationship to bicarbonate ion concentration or DIC. For instance, barium would be an ideal candidate, as it is physiologically inert as is Sr (Salisbury and Ross 1992), which for instance excludes magnesium as a test candidate. Unfortuantelly, barium could not be measured in the setup used, as concentrations in the shell were too low. Similar experiments to the ones described here, maybe under elevated barium concentrations could be used to test the here-proposed model.

4.5. Sr/Ca-DIC sensitivity and paleoceanographic implications

The here studied species, *A. aomoriensis*, is a shallow water benthic foraminifer and rarely used for palaeooceanographic reconstructions. However, it is ideally suited for our study, since it is adapted to an environment where carbonate chemistry frequently changes. This makes it likely that observed effects under experimental conditions as extreme as imposed here are not laboratory artefacts. The element composition (e.g. Mg/Ca and Sr/Ca) of *Ammonia* spp. is similar to that of many other rotalid species. Moreover, the dependency of element partitioning on environmental conditions (e.g. Mg incorporation as a function of temperature) is similar for many of these low-Mg perforate species (Wit et al., 2012; Toyofuku et al., 2011), which allows translation of our results to other benthic species. Inter-species differences in published calibrations, however, make it challenging to apply our results directly to genera other than *Ammonia* and derive accurate [DIC] reconstructions from benthic foraminiferal Sr/Ca values in general.

Despite this cautionary note, our described dependency of Sr/Ca to DIC may help explain reported inter-ocean differences in Sr/Ca of *Cibicidoides wuellerstorfi* (Yu et al., 2014). Based on the average DIC concentrations in the deep Pacific and deep Atlantic, our calibration (Fig. 1) would predict a slightly larger difference in Sr/Ca than reported by Yu and co-workers. Such a mismatch in absolute Sr/Ca differences may, however, be caused by potential inter-species differences in Sr/Ca-DIC calibrations.

Stoll and Schrag (1998) have estimated that mean ocean Sr/Ca may change between 1 and 3 % as a result of changing sea levels over glacial-interglacial cycles. Indeed, Martin and coworkers (2000) reported systematic downcore oscillations in foraminiferal Sr/Ca over these cycles, which were consistent between ocean basins, for both planktonic and benthic species. The amplitude in Sr/Ca change was 4-6% maximum and could not be correlated to dissolution or temperature effects. The authors discussed possible effects of salinity and pH, and although their attributed changes were too small (2% relative) to account for the full change, they pointed into the right direction of change. The authors therefore assigned the rest of the change to G-IG changes in mean ocean Sr/Ca.

Glacial-interglacial changes in carbonate chemistry are based on the well-documented increase in pCO_2 during termination 1 from 180 to 280 µatm (Petit et al., 1999) and a subsequent decrease in open ocean surface pH from 8.3 to 8.1 (Hönisch et al., 2009). Using

the software package CO2SYS (Pierrot et al., 2006), this increase in pCO_2 resulted in an estimated increase in DIC of 130 µmol/kg. Using our correlation between DIC and foraminiferal Sr/Ca (Fig. 1), this should have resulted in an increase in Sr/Ca_{cc} of 1.2% (from 1.268 to 1.284 mmol/mol). This change is, however, too small to account for the reported 2-4% change in foraminferal Sr/Ca (Martin et al., 2000). Allen and coauthors (2016) have performed a similar DIC calibration study with the planktonic species *Globigerinoides sacculifer* and found a slope between Sr/Ca_{cc} and DIC twice as step as we found for *Ammonia* sp. Applying this linear regression equation would lead to a change in Sr/Ca_{cc} between G and IG of approximately 3%, which is in the order of the reported average Sr/Ca_{cc} changes over glacial-interglacial cycles (Martin et al., 2000). While the direction of the Sr/Ca-DIC calibration very likely is the same for different species (assuming similar biomineralization controls), the slope of the calibration may well vary between species (cf those for Mg/Ca and Temperature, see Regenberg at al., 2009 for an overview). This means that the absolute change in foraminiferal Sr/Ca as a response to the same environmental change will vary between species. Future development of foraminiferal Sr/Ca as a proxy for seawater DIC will have to show whether glacial-interglacial changes in carbonate chemistry can fully explain observed changes in planktonic foraminiferal Sr/Ca.

Different trace elemental calibrations have been proposed in the literature for several C-system parameters. This dataset is based on the same samples and analyses published in Keul et al. (2013b), where the dependence of U/Ca on carbonate ion concentration has been described. A major strength of trace elemental analyses via ICP-MS lies in the fact, that several elements can be analyzed simultaneously on the same single foraminiferal chamber. When combining Sr and U analyses on the same samples, the full C-chemistry can be calculated employing the regressions giving in this paper and in Keul et al (2013b). A combination with e.g. Mg/Ca and oxygen isotopes can allow for the calculation of temperature and salinity. In order to achieve these multi-proxy reconstructions, more culturing studies are needed to fully characterize the uncertainties associated. Multi-proxy reconstructions can then be used to correct for the mutual effects of trace elements and isotopes on each other.

5. Conclusions

In the field, potentially many environmental (i.e. target) parameters change in concert, making it rarely straight forward to isolate a single influence on a particular proxy. Therefore culture experiments provide a very useful approach: in the present study we not only identified seawater carbonate chemistry as an influence on Sr/Ca, but also narrowed the options down to bicarbonate ion concentration (DIC) in the benthic foraminifera *Ammonia sp*. Applying the DIC calibration to a published dataset of paleo-Sr/Ca supports the validity of Sr/Ca as a carbonate system proxy. Furthermore, our study also shows that more research is needed to fully understand the Sr-incorporation in deep sea species growing at different depths. We present a conceptual model that proposes how Sr is incorporated in foraminiferal calcification during biomineralization. Taking this model a step further and use it to explain multiple, environmental impacts on Sr/Ca requires both more experimental and field calibrations, as well as a more comprehensive understanding of foraminiferal biomineralization. For example, the ion transporters responsible for the influx of DIC (bicarbonate transporters, carbonate ion transporters, CO_2 diffusion) and Ca (seawater leakage, transmembrane transport) remain poorly understood.

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Figure captions

Figure 1: Foraminiferal Sr/Ca versus individual C-system parameters: a) pCO_2, b) bicarbonate ion concentration, c) carbonate ion concentration, d) TA, e) DIC, and f) pH. Individual laser-ablation measurements are represented by **x** and +, whereas squares indicate mean values. Blue represents data from TA manipulation (A1-A4) and pink indicates pH-stable manipulation (B1-B4). Lines represent linear regression lines for Sr/Ca versus the respective C-system parameter for the TA- and pH-stable manipulation separately. Due to the relatively small range in x-axis variations in the correlation between Sr/Ca and DIC in the TA manipulation (blue, e), the significance of regression is confined and regression slope has been omitted.

Figure 2: Foraminiferal D_{Sr} versus individual C-system parameters: a) pCO_2, b) bicarbonate ion concentration, c) carbonate ion concentration, d) TA, e) DIC, and f) pH. Individual laser-ablation measurements are represented by **x** and +, whereas squares indicate mean values. Blue represents data from TA manipulation (A1-A4) and pink indicates pH-stable manipulation (B1-B4). Lines represent linear regression lines for Sr/Ca versus the respective C-system parameter for the TA- and pH-stable manipulation separately. Due to the relatively small range in x-axis variations in the correlation between Sr/Ca and DIC in the TA manipulation (blue, e), the significance of regression is confined and regression slope has been omitted.

Figure 3: Foraminiferal Sr/Ca versus Omega Individual laser-ablation measurements are represented by **x** and +, whereas squares indicate mean values. Blue represents data from TA manipulation (A1-A4) and pink indicates pH-stable manipulation (B1-B4). Lines represent linear regression lines for Sr/Ca versus the respective C-system parameter for the TA- and pH-stable manipulation separately.

Figure 4: Schematic model of the processes during calcification. Calcite precipitation is thought to occur in a confined space ("delimited biomineralization space", DBS), which is created by the pseudopodial network (PN). Calcium carbonate (CC) is precipitated onto a template ("primary organic sheet", POS). A) The uptake of trace elements and its consequent incorporation into the calcite consists of two steps (inset in A): A1) Uptake from seawater into the DBS, where A2) the trace element is then incorporated into the calcium carbonate. During the uptake of Ca, small amounts of Sr also enter the DBS and consequently the CC. B1) High DIC in the culturing media leads to high CO_2 and HCO_3^- concentrations. B2) Both of these enter the DBS, where they are converted to CO_3^{2-}, due to the high pH inside the DBS. The red arrows depict this increase. B3) The foraminifera has to decrease Ca (red arrow) in order to keep ▸ constant. B4) The decrease of Ca inside the DBS causes Sr/Ca in the DBS to increase and consequently also in the calcite (red arrow), which is depicted graphically in B5).

Figures

Figure 1

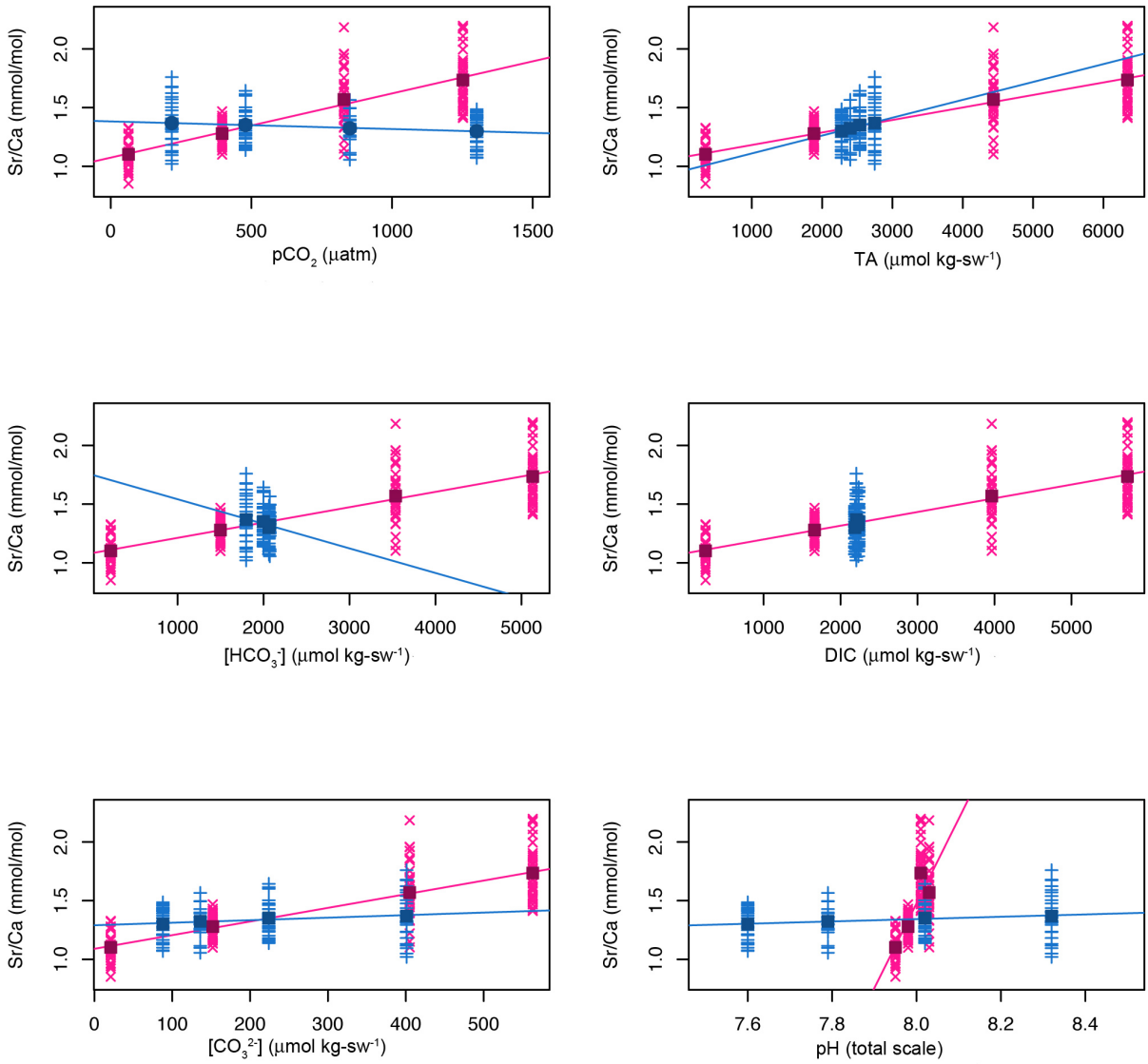


Figure 2

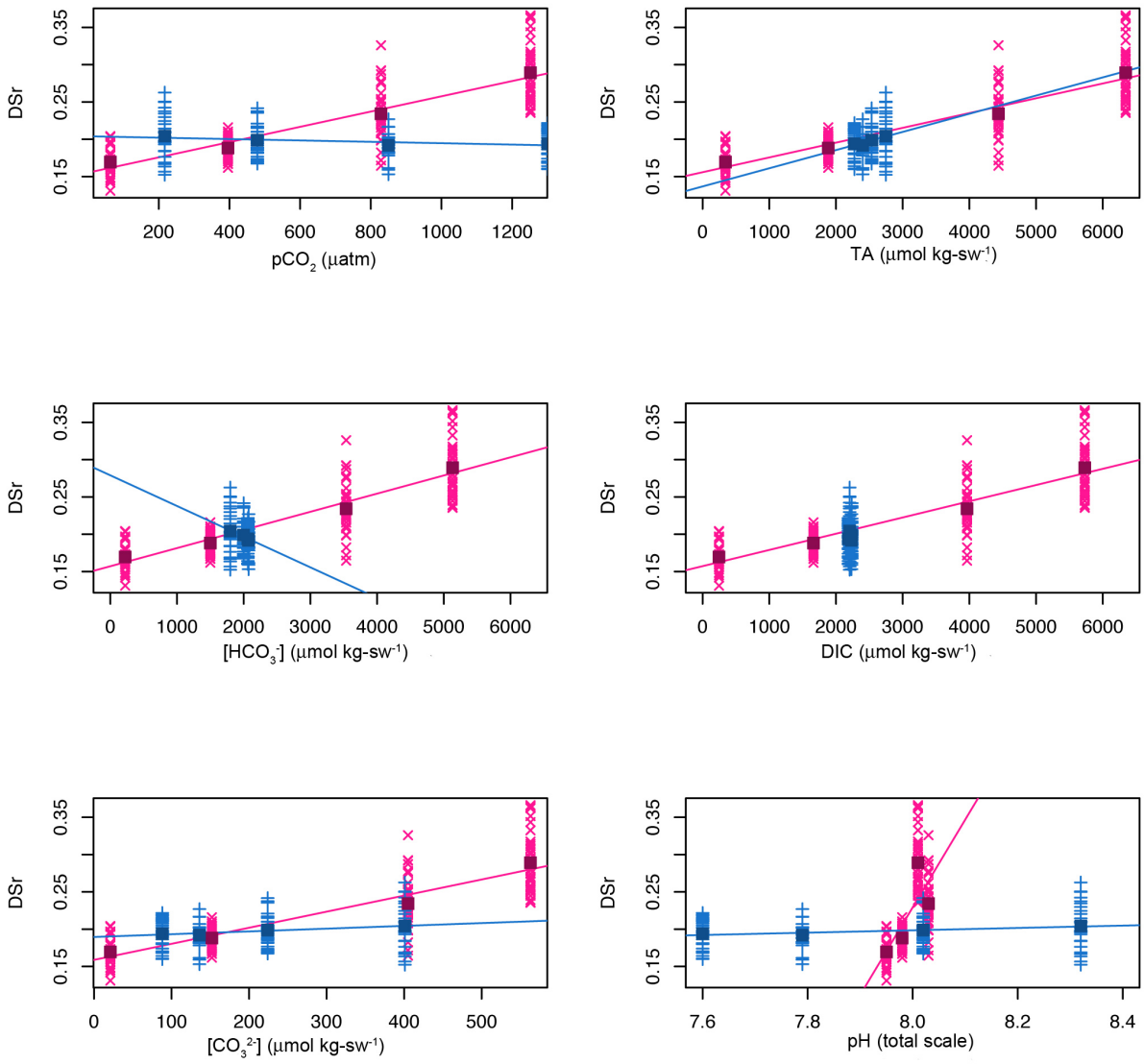
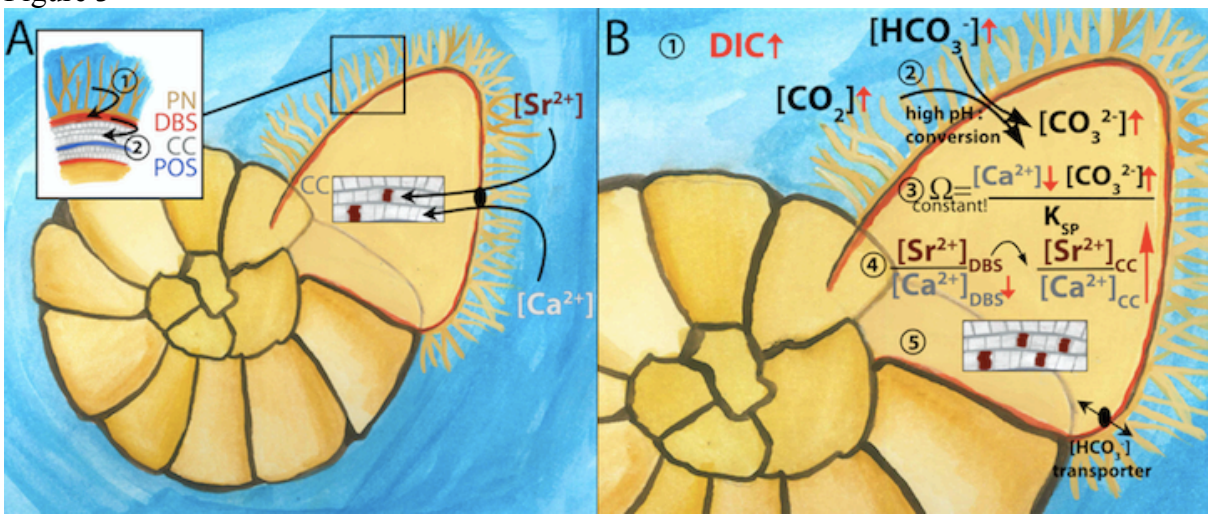

DSr
0.15
0.25
0.35
200
400
600
800
1000
1200
pCO2 (µatm)
0
1000
2000
3000
4000
5000
6000
TA (µmol kg-sw-1)
[HCO3-] (µmol kg-sw-1)
DIC (µmol kg-sw-1)
100
300
500
[CO32-] (µmol kg-sw-1)
7.6
7.8
8.0
8.2
8.4
pH (total scale)

Figure 3



853
854
855

856

Figure 4

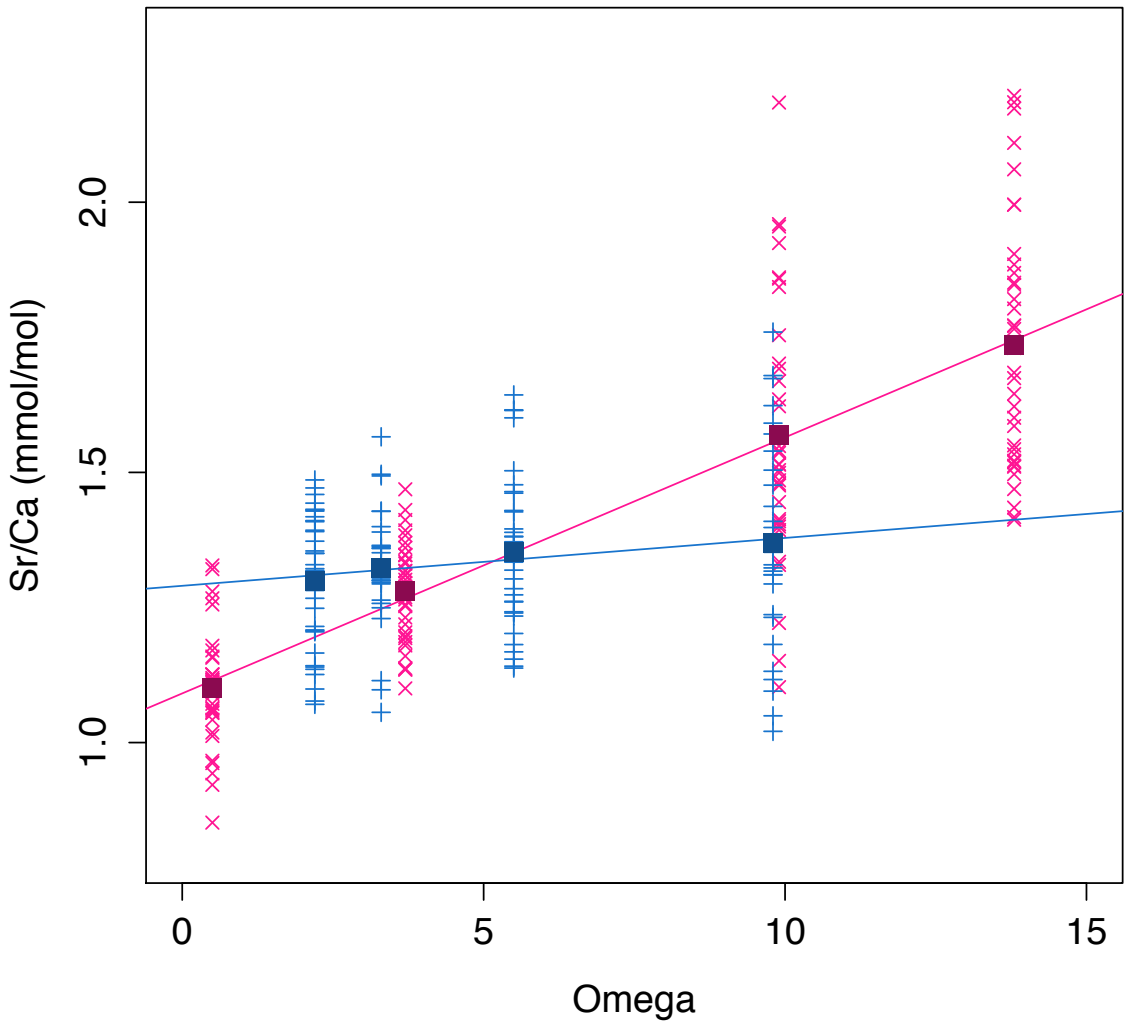


Table captions

Table 1: C-system parameters, Sr/Ca, DSr (calculated partition coefficient) and growth rates. DIC and pH of the seawater media were measured and used as input parameters to calculate the other C-system parameters using the CO2SYS software (Pierrot et al., 2006). Salinity and temperature were used as additional input parameters. pCO_2 values supplied from the gas-mixing system are additionally listed ("nominal"). Stability was checked over the course of the experiment by regular pH measurements and control of the pCO_2 provided by the gas-mixing system (precision approximately 10 µatm). Sr/Ca of the seawater media (sw) and calcite (cc) are listed as well as calculated partition coefficient D_{Sr} for TA manipulation (A1–A4) and pH-stable manipulation (B1–B4) treatments. $\Omega_{cc}=[Ca^{2+}]* [CO_3^{2-}]/K_{sp}$ (K_{sp}= solubility constant).

Table 2: Linear regression analyses between foraminiferal Sr/Ca and respective C-system parameters. Listed is the intercept with the y-axis and the slope together with the associated standard errors (SE) for these parameters. Furthermore, p-value, F-value and R^2 of the regression analyses are given.

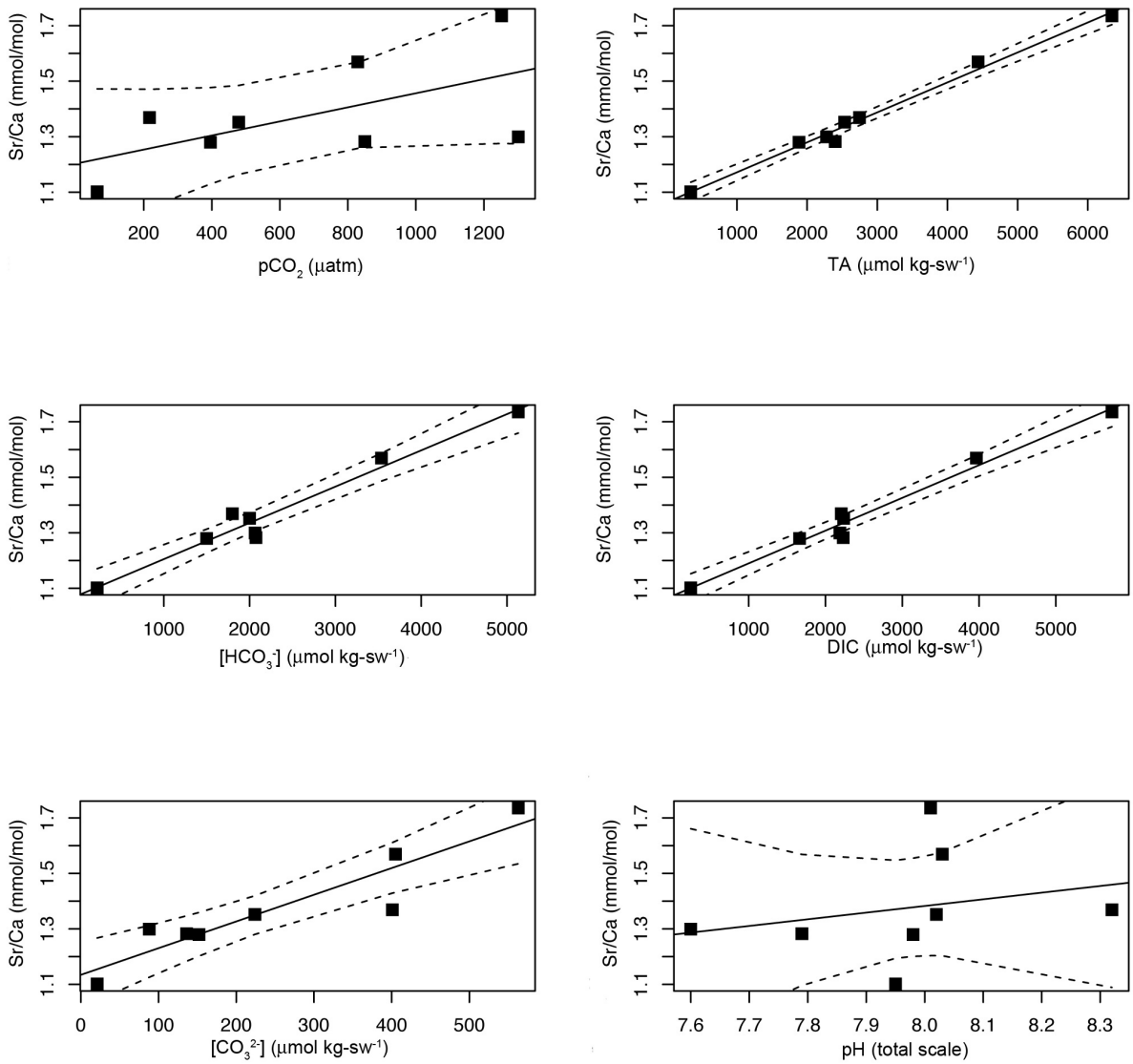
Table 1

	Treatments							
	A1	A2	A3	A4	B1	B2	B3	B4
pCO_2 (µatm) "nominal"	180	380	950	1400	180	380	950	1400
pCO_2 (µatm)	217	479	850	1301	63	396	829	1252
CO_3^{2-} (µmol/kgSW)	401	224	136	88	21	152	405	563
HCO_3^- (µmol/kgSW)	1798	1999	2073	2063	223	1499	3536	5131
DIC (µmol/kgSW)	2205	2236	2232	2187	246	1662	3965	5729
TA (µmol/kgSW)	2747	2535	2400	2277	342	1884	4436	6343
pH total scale	8.32	8.02	7.79	7.60	7.95	7.98	8.03	8.01
Ωcc	9.8	5.5	3.3	2.2	0.5	3.7	9.9	13.8
Salinity	32.8	32.8	32.8	32.8	32.7	32.7	32.8	32.6
Sr/Ca sw (mmol/mol)	6.7	6.8	6.9	6.7	6.5	6.8	6.7	6.0
Sr/Ca cc (mmol/mol)	1.4	1.4	1.3	1.3	1.1	1.3	1.6	1.7
DSr	0.20	0.20	0.19	0.19	0.17	0.19	0.23	0.29
Growth rate (10^{-2} µg/µm)	11.2	7.3	7.6	9.7	6.0	9.3	12.8	9.0

Table 2

	all 8 treatments combined						
	Intercept	SE	slope	SE	p	R^2	F
pCO_2 (µatm)	1.7E-01	4.4E-03	4.9E-05	5.4E-06	<2e-16	0.23	83.5
CO_3^{2-} (µmol/kgSW)	1.6E-01	3.0E-03	1.9E-04	9.9E-06	<2e-16	0.56	355.5
HCO_3^- (µmol/kgSW)	1.5E-01	3.1E-03	2.5E-05	1.2E-06	<2e-16	0.62	458.4
DIC (µmol/kgSW)	1.5E+00	3.1E-03	2.2E-05	1.0E-06	<2e-16	0.63	476.6
TA (µmol/kgSW)	1.5E-01	3.1E-03	2.1E-05	9.3E-07	<2e-16	0.64	490.8
pH total scale	-1.8E-01	1.1E-01	4.8E-02	1.4E-02	<0.001	0.04	12.4
Ωcc	1.6E-01	3.0E-03	7.6E-03	4.0E-04	<2e-16	0.56	357.7

Supplementary Figures



S1: 95% Confidence Intervals for the regressions presented in Table 2.