Dear editor,

We kindly thank Joachim Schönfeld, Janett Voigt, Ed Hathorne and the anonymous reviewer for their constructive comments on the manuscript. We have uploaded a revised version of our manuscript that takes into account all comments by the reviewers. Below, we have listed our answers to all of their issues. The comments by Dr. Schönfeld were addressed in a previous reply and will not be repeated here. We hope you will consider this version of our manuscript for publication in Biogeosciences.

J.C. Wit and co-authors

Review by J. Voigt and E.C. Hathorne

"Some import details are missing from the methods section, e.g. how alkalinity and DIC have been measured and that an Excimer laser system was coupled to an Element2? Also were the foraminifera cleaned at all before LA-ICP-MS analysis? Using NaOH buffered H2O2?"

We agree with the reviewers that including this information improves the manuscript and we have therefore extended the methods section in the revised version of our manuscript.

> Calcitic Na/Ca, Sr/Ca and Mg/Ca were determined using deep Ultra Violet wavelength (193nm) laser ablation (LA) (Geolas 200Q Excimer) coupled to a sector field Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Element 2, Thermo Scientific), at Utrecht University. Prior to LA-ICP-MS measurements, foraminiferal tests were cleaned in pH buffered 5% sodiumhypochloride (NaOCl) for 10 min to remove organic material and rinsed twice in ultra pure (MilliQ) water. Individual foraminifera were subsequently rinsed in 0.5 ml Eppendorf cups with ultra pure (MilliQ) (3 times), methanol (2 times), ultra pure (MilliQ) (3 times) and dried before laser ablation analyses.
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> Alkalinity was determined by automated titration of 50 ml of sample water with a weak acid solution (0.1 M HCl). Subsamples (10 ml) for DIC were filtered over a 0.2 μm mesh and measured on an automated Shimadzu TOC 5050 (TIC method, ± 2 μmol/l, Utrecht University).

"Figure 1 describes a correlation between test size and salinity and it seems possible between the Na/Ca ratio and salinity simply reflects the correlation of Na/Ca with test size. Therefore, it would be useful to demonstrate a correlation of Na/Ca ratio with salinity for specimens with similar test size to fully exclude any size effects."

We agree with the reviewers that this would improve the interpretation of our results. Therefore, we added a figure (no 4) on the relation between salinity and Na/Ca within each of the four treatments. However, the amount of data per salinity experiment is not sufficient to divide the dataset into narrow size ranges and thus fully exclude an ontogenetic effect on Na/Ca. This is mainly due to the relatively high inter-individual variability in Na/Ca. Because differences in size might reflect growth rate (see also comments by anonymous reviewer #1), we added the Sr/Ca and D_{Sr} data for our experiment. For the current experiments incorporation of Sr into calcite may primarily reflect calcite precipitation rates, since other parameters controlling Sr/Ca in foraminifera (i.e. Temperature, Ω) were kept constant. Since Sr/Ca does not vary with size (figure 4), it is unlikely that calcite precipitation rates (not to be confused with foraminiferal growth rates) varied over the foraminifer's lifetime. Although this does not fully exclude a small growth rate effect, this makes an ontogenetic effect on the

incorporation of Mg and Na less likely.

Incorporation of certain elements into the calcite of some foraminiferal species is correlated to ontogeny, i.e. depending on test size (Nürnberg et al., 1996; Wit et al., 2010; Dueñas-Bohórquez et al., 2011b). Since test size of the cultured foraminifera varied between salinities (Figure 1, Table 3), the relation between foraminiferal Na/Ca and salinity may potentially have been affected by differences in test sizes. Incorporation of Sr into foraminiferal calcite has previously been linked to changes in growth rates and size in both inorganic calcite precipitation experiments (Lorens, 1981, Mucci and Morse, 1983, Nehrke et al., 2007) and foraminiferal culture and core top studies (Elderfield et al., 2002, Kisakurek et al., 2008, Dissard et al., 2010) and can therefore be used to unravel size effects from salinity control on Na incorporation. Average Sr/Ca values decrease slightly as size increases (Figure 2) and salinity decreases (Figure 3). To determine whether size affected Sr/Ca, we analyzed the relation between size and Sr/Ca within each experiment (i.e. at constant salinity, Figure 4). No significant correlation between size and Sr/Ca was found within individual experiments. We, therefore conclude that, despite the variation in foraminiferal size, calcite precipitation rates (i.e. the rate at which $CaCO_3$ precipitates during chamber formation) were similar and hence did not affect our Na/Ca-salinity calibration.

"Page 6044 line 9 ". . .test size significantly increased with salinity. . ." but both Figure 1 and Table 3 clearly indicate a decrease in test size with increasing salinity."

This has been adjusted in the revised manuscript.

"Page 6044 line 11-14 ". . .and of the same order in single chamber ablation profiles." This is confusing especially when the methods state "Elemental ratios. . .were based on the average of each ablation profile" and no ablation profiles, time (cha mber wall depth) resolved data, are presented. It would be nice to see an additional figure showing the intra-shell wall variability observed for Na/Ca and the relationship to Mg/Ca."

A figure (Figure 1) containing representative examples of the intra-shell variability in Na/Ca, Mg/Ca and Sr/Ca has been added to the manuscript.

Anonymous Reviewer 1

"1- (Minor) –Methods-page6042-lines25-27 Carbonate system is very difficult to maintain during the culturing experiment. All discussed data can completely have different meaning if, for example, pH was drifting significantly during the experiments as it will have major implication for foraminiferal Me/Ca values. The authors stated that they monitored it during the experiment and found it constant. This statement has to be accompanied by data. At minimum they will need to provide DIC, alkalinity, pH or pCO2 at the beginning and at the end of each experiment. If they monitored it then it would be best to provide a time series plot."

We added a table (Table 2) to the manuscript describing the values and standard deviations of all measured parameters over the course of the experiment. Parameters were determined at the start and end of each experiment and twice during the experiment.

Experiment	Salinity	Temperature (°C)	DIC (µmol/kg)	Alkalinity (µmol/kg)	CO_3^{2-} (µmol/kg)	$\Omega_{Calcite}$	Mg/Ca (mol/mol)	Na/Ca (mol/mol)	Sr/Ca mmol/mol)
S 30.0	30.0 ± 0.1	20.1 ± 0.3	2131 ± 17	2462 ± 32	246 ± 15	6.10 ± 0.4	4.98 ± 0.02	46.97 ± 0.48	5.01 ± 0.08
S 32.5	32.5 ± 0.2	20.0 ± 0.2	2222 ± 25	2543 ± 46	238 ± 50	5.81 ± 1.2	5.08 ± 0.03	47.82 ± 0.69	9.27 ± 0.15
S 36.1	36.1 ± 0.2	19.9 ± 0.1	2188 ± 24	2526 ± 23	244 ± 32	5.80 ± 0.8	5.15 ± 0.04	48.76 ± 0.89	9.38 ± 0.15
S 38.6	38.6 ± 0.1	20.0 ± 0.2	2126 ± 7	2493 ± 21	258 ± 40	6.01 ± 0.4	5.19 ± 0.04	48.95 ± 0.44	9.55 ± 0.15
µ		20.0	2161	2502	248	5.97	5.10	48.12	8.30
σ		0.4	41	40	13	0.44	0.09	0.91	2.20

"First, authors used NIST 610 for cross calibration but I am not sure this is a safe approach especially as they used different energy outputs during ablation of the standard. Note that there is _14% of Na2O in the NIST610 and only few hundreds of ppm in foraminifera where there is already huge matrix differences, and on top the standard is ablated with 5 times more energy. NIST610 is ok for Mg/Ca in forams but Na/Ca is a risky and unproven approach."

The reviewer is right that switching between glass and calcium carbonate might introduce extra uncertainty when calculating foraminiferal Na/Ca. However, calibration of element/calcium ratios in calcium carbonate samples using a NIST glass standard has been demonstrated to be accurate for many elements when using a 193 nm laser (Hathorne et al., 2008), despite switching between ~1 and ~5 J/cm^2 (e.g. Dueñas-Bohórquez et al., 2011) Although 13.4% (m/m) of NIST610 is Na_2O, 10% is Na (m/m), which is ~50 times more than the abundance in foraminiferal $CaCO_3$. Still, ICP-MS is very well suited for calibrations over several orders of magnitude. The range offset is similar to that of Mg in NIST610 versus foraminiferal calcite, making the NIST610 well suited for Na/Ca calibrations.

"Second, there is potential ICP front-end contamination (e.g., cones, injector) with Na and drift in signal-to-background ratio. In normal practice, we for example, have specially dedicated 'low-Na' ICPMS cones for measuring Na/Ca in biogenic calcite. This is because after introduction samples with high Na concentrations (like NIST610) the Na background will be higher than the foraminiferal signal. And worse, this background is increasing in each consecutive high-Na sample, which will degrade the quality of Na/Ca measurements. So I am puzzled how the authors managed to measure NIST610 and foraminifera using the same ICP front-end. What is the signal-to-background ratio on the forams samples? Was it constant through time (throughout the experiment)?"

Using the NIST610 may introduce contamination to especially the sample cell (dust) and hence impact the foraminiferal Na/Ca. For this reason, we measure 30-60 seconds of background before and after each sample (forams as well as glass). This allows correcting for any changes in Na-background during a day of measuring by using the background Na-values that are associated with each measurement individually. At the start of the laser ablation dust from the sample chamber could also be potentially remobilized, contaminating the start of the profile. This was carefully checked for, but no appreciable carry-over was detected. Moreover, drift in Na (as well as all other elements analyzed) background was monitored throughout the analyses. We added an excel file to show the difference in background and signal Na counts over a day of measuring. The selected profiles were part of a sequence with >100 samples. In summary, background values for Na of a NIST610 at the beginning of the day were ~215k cps and ~230k cps before and after the ablation of the glass, so that measuring the NIST610 increased background Na counts by ~7%. At the end of the day, background values increased up to 330k – 370k cps (before and after ablation, respectively), which is an increase of ~12% (Figure 1). The associated background to noise ratios were similar for these measurements (~550). Background Na-values for foraminiferal samples were similar and increased marginally over the course of a day, and hence did not affect precision of Na/Ca.

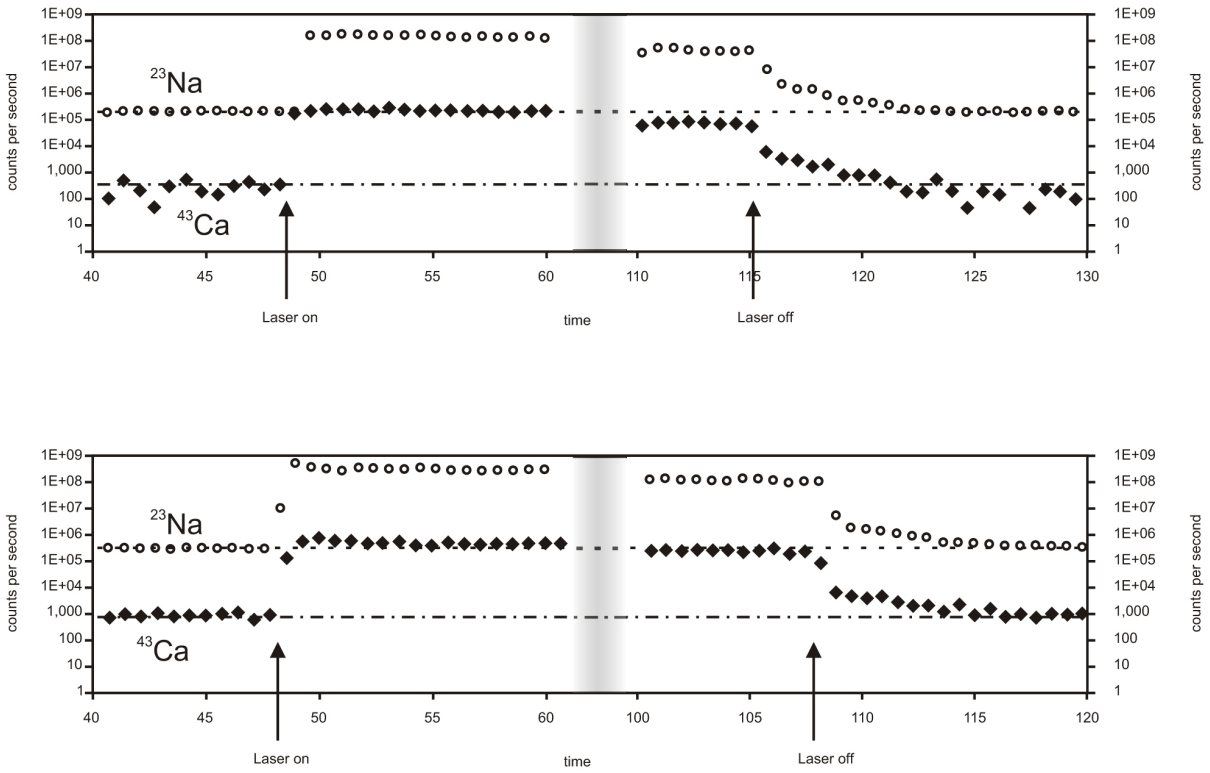

Figure 1: Sodium and calcium background counts for two N610 Glass standards. Upper standard was measured at the beginning of the day, while the lower standard was measured at the end of the day. NB. Note rapid washout also for Na.

"There is very limited information given about Me/Ca composition of culturing media. Surprisingly, Na/Ca (value?) of seawater is missing. The authors have Dna so they should have Na/Ca which has to be provided. It is also important to understand if seawater Na/Ca affects foraminiferal Na/Ca. Note salinity is not = Na/Ca because there can be changes in Ca concentration. Were Na/Ca and Ma/Ca values constant during the culturing experiment? Note authors are dealing with chemical cocktail and inorganic precipitation is possible, which is sometimes difficult to identify by simple visual examination."

We have added the elemental concentration for Mg/Ca, Sr/Ca and Na/Ca to table 2. Constant concentrations show that no inorganic precipitations occurred.

"There is no description on how the shells were cleaned prior to geochemical analyses. The main focus of this work is Na/Ca and seawater contains high amounts of Na and therefore shell contamination is expected. How did the authors clean their shells and how did they make sure they are cleaned well?"

We added a more detailed description on cleaning the foraminiferal tests to the manuscript (see also answer to review by Voigt and Hathorne).

"Results section is only half page long!!! This is a research paper with big claims and only half page results??? I think this is unacceptable and in fact the lack of results became quickly obvious in the discussion. To my knowledge, this first paper is describing Na/Ca in forams using LA-ICPMS. So far, there is limited information in the literature on how Na is distributed within the foram shells and how it is different in different chambers. The authors clearly have this information but failed to present it. Arguably, it is not vital in the context of this manuscript but in my opinion this is a MUST HAVE information, which can help interpret some of the correlations identified in this work. I strongly suggest adding a few profiles of Na/Ca and chamber comparison. Also, it would be nice to see Na/Ca profile/chamber values correlated with Mg/Ca."

We appreciate the eagerness of the reviewer to see more detailed results and have tried to accommodate this as much as possible. Also in relation to some of the earlier comments, we have now added a number of figures, including a number of LA-profiles and data on the (changes in) background Na values. We have also added the raw elemental data to the manuscript to allow additional (statistical) analysis. We fully agree that the present manuscript only sets the stage for much future work that is needed to clarify the incorporation of Na in foraminiferal test calcite.

"On few occasions, the authors discussed Na/Ca banding in forams and refer to Erez 200x. I am a bit confused here because if I remember correctly I have not seen (published) a single profile of Na/Ca across foraminifera. Definitely Jonathan Erez has none of them. Are the authors referring to their findings? I think for this discussion the authors need a separate section in the results.The same goes for the authors comment on lack of 'hot-spot' distribution of Na/Ca across the shell. Please present it in the results".

We adjusted the manuscript and the discussion and show Na/Ca distribution through the shell in figure 1. There is some information on Na/Ca in foraminiferal calcite in the Erez (2003) paper (page 130) describing a heterogeneous distribution of Na in the test wall. The reviewer might have missed this in the Erez paper as there are not much analytical details on how Na was measured, nor is there a figure showing the Na/Ca of these profiles. We suggest, however, to keep the reference to Erez' paper and his claim that Na may not be as homogenously distributed as it looks in our specimens. This difference may be the result of symbiont activity in their measured species (*A. lobifera*) versus its absence in our *Ammonia* spp., in the same way Mg may be heterogeneously distributed in the spherical chamber walls of *O. universa* while it appears homogenously distributed in (benthic) foraminifera that have no symbionts.

> Both Mg and Na showed distinct banding in the test carbonate of some species (Erez, 2003) and changes in the relative contributions of high and low Na and Mg bands could explain the observed inter chamber variability. The source of these bands has been attributed to vertical movements of individual foraminifera, although the magnitude of the variability and its presence in planktonic as well as benthic species renders this unlikely due to the small in-sediment temperature variability (Sadekov et al., 2008, Wit et al., 2010). Another potential source of these bandings is the periodical change in the carbonate ion concentration. Light-Dark cycles have been reported to influence the activity of photosynthetic symbionts in planktonic foraminifera which in turn effects the carbonate ion concentration of its micro-environment and thereby the incorporation of Mg (Eggins et al., 2004). These same variations have been observed in benthic foraminifera as a result of carbonate ion concentration changes with in sediment habitat depth of the foraminifera (Elderfield et al., 2006, Rathmann and Kuhnert, 2008). Alternatively, such banding has been ascribed to variable element adsorption to organic linings and elemental differences between primary and secondary calcite (Erez, 2003). This variability potentially hinders the accuracy of proxies based on the Na/Ca and Mg/Ca values of foraminiferal calcite, but does not impact their applicability in paleoceanography when sufficient specimens are combined to determine element/Ca values to account for the inter- and intra-individual variability in element/Ca ratios (Sadekov et al., 2008, Wit et al., 2012).

"The authors found a strong intercorrelation between shell size, Na/Ca, Mg/Ca and seawater. Although I understand that establishing a salinity proxy is the primary goal of this study I think the dataset, as it stands, can be interpreted differently. One of the striking features of the dataset is the strong correlation of Me/Ca not only with salinity but also with shell size. The authors argue in the discussion that ontogenetic effect, to which they attributed shell size, by itself does not have effect on foraminiferal Me/Ca. However, a major potential factor, such as calcification rate, was not considered. Shell size is potentially a direct measure of calcification rate, which has a huge (orders of magnitude) effect on trace metal incorporation into calcite. It has been argued for ages that the relationship between Mg/Ca values of foraminifera or ostracoda and their shell size is related to calcification rate. Also, there are numerous inorganic experiments, which clearly establish the link between Me/Ca and calcification/precipitation rate. Therefore, it is quite possible that the increase in Me/Ca reported in this work is due to decrease in calcification rate in experiments with higher salinities. In fact, it may not be related to salinity at all but to concentration of Ca in seawater, for

example. I may be wrong but these potential problems have to be one of the major points of the discussion. Please also note that the argument used by the authors in discussing ontogenetic effect (i.e. individual shell size vs Na/Ca value) is incorrect. Processes, which controls Me/Ca at individual shell level and bulk/average Me/Ca level, could be different. For example, at individual level there is no correlation between individual shell Mg/Ca values and temperature (just biological nose) but the average Mg/Ca value of 20-30 shells has trong correlation with temperature. And again, it is not ontogenetic changes but calcification rate which is primary the driving force behind shell size effect. I would like to suggest a few hints, which may be helpful for the authors in their rebuttal. Try to estimate thickness of the shells, which would be a more accurate measure of calcification rate. Use either LA profile or SEM measurements of few shells if they are still available. Compare this with culturing logbook and life span of each foraminifera. Use Sr/Ca ratio. Sr was also measured as mentioned in the method section. It could help the argument because Sr response to calcification rate is opposite to that of Mg."

The suggestion to include Sr/Ca to check for changes in precipitation rates was followed and showed no sign of change with size, suggesting that the calcite precipitation rates do not vary significantly with age and size. Moreover, the variability in Na/Ca (and Mg/Ca) is not related to variability in Sr/Ca (single chamber Sr and Na for the whole dataset are not correlated), hinting to a minor impact of precipitation rate on Na-incorporation. We have added the above arguments to the revised version of our manuscript. However, we also added one sentence to point out that growth rate and rate at which calcite precipitates during the addition of a single chamber are not necessarily correlated.

> Incorporation of certain elements into the calcite of some foraminiferal species is correlated to ontogeny, i.e. depending on test size (Nürnberg et al., 1996; Wit et al., 2010; Dueñas-Bohórquez et al., 2011b). Since test size of the cultured foraminifera varied between salinities (Figure 1, Table 3), the relation between foraminiferal Na/Ca and salinity may potentially have been affected by differences in test sizes. Incorporation of Sr into foraminiferal calcite has previously been linked to changes in growth rates and size in both inorganic calcite precipitation experiments (Lorens, 1981, Mucci and Morse, 1983, Nehrke et al., 2007) and foraminiferal culture and core top studies (Elderfield et al., 2002, Kisakurek et al., 2008, Dissard et al., 2010) and can therefore be used to unravel size effects from salinity control on Na incorporation. Average Sr/Ca values decrease slightly as size increases (Figure 2) and salinity decreases (Figure 3). To determine whether size affected Sr/Ca, we analyzed the relation between size and Sr/Ca within each experiment (i.e. at constant salinity, Figure 4). No significant correlation between size and Sr/Ca was found within individual experiments. We, therefore conclude that, despite the variation in foraminiferal size, calcite precipitation rates (i.e. the rate at which $CaCO_3$ precipitates during chamber formation) were similar and hence did not affect our Na/Ca-salinity calibration.

"The section on Mg correction is completely out of place in my opinion. First, effect of salinity on Mg/Ca is still debated and by itself controversial. Second, this section is badly structured and has many controversial and subjective statement/arguments (e.g. Mg/Ca seawater not affecting Mg/Ca in forams; calcification pathways; individual shell Mg/Ca correlated with size at one salinity but does not in others). And last, it is absolutely not applicable for paleorecords. Let us assume that things work as suggested. Changes in paleosalinity were usually 1-2% which would be 5-10% in Mg/Ca values and is equivalent of 0.5-1C. So in theory, we should correct these very small changes using Na/Ca values. However, error bars on Na/Ca and Ma/Ca are already larger than this, plus there are uncertainties associated with calibration curves (e.g Mg/Ca vs. T and Salinity plus Na/Ca vs. salinity). So if errors are correctly propagated through calculation 'corrected' temperature, estimates will be much worse (more uncertain) than initial temperature estimates."

We agree with the reviewer that this section distracts from the general flow of the paper and omitted the section from our manuscript.