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Research paper

Variability in calcitic Mg/Ca and Sr/Ca ratios in clones of the benthic foraminifer *Ammonia tepida*

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ABSTRACT

Biological activity introduces variability in element incorporation during calcification and thereby decreases the precision and accuracy when using foraminifera as geochemical proxies in paleoceanography. This so-called 'vital effect' consists of organismal and environmental components. Whereas organismal effects include uptake of ions from seawater and subsequent processing upon calcification, environmental effects include migration- and seasonality-induced differences. Triggering asexual reproduction and culturing juveniles of the benthic foraminifer *Ammonia tepida* under constant, controlled conditions allow environmental and genetic variability to be removed and the effect of cell-physiological controls on element incorporation to be quantified. Three groups of clones were cultured under constant conditions while determining their growth rates, size-normalized weights and single-chamber Mg/Ca and Sr/Ca using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). Results show no detectable ontogenetic control on the incorporation of these elements in the species studied here. Despite constant culturing conditions, Mg/Ca varies by a factor of ~4 within an individual foraminifer while intra-individual Sr/Ca varies by only a factor of 1.6. Differences between clone groups were similar to the intra-clone group variability in element composition, suggesting that any genetic differences between the clone-groups studied here do not affect trace element partitioning. Instead, variability in Mg/Ca appears to be inherent to the process of bio-calcification itself. The variability in Mg/Ca between chambers shows that measurements of at least 6 different chambers are required to determine the mean Mg/Ca value for a cultured foraminiferal test with a precision of ≤10%.

1. Introduction

Incorporation of various trace elements into foraminiferal calcite is affected by environmental parameters and therefore, calcitic element/calcium ratios are widely used to reconstruct marine paleoenvironments. For example, foraminiferal Mg/Ca ratios have been shown to be primarily correlated with seawater temperature (e.g. Nürnberg et al., 1996) and are used in combination with test calcite $\delta^{18}O$ to reconstruct paleo-seawater $\delta^{18}O$ (e.g. Elderfield and Ganssen, 2000; Lear et al., 2000), and estimate salinity. Elevated Mg-incorporation into calcite at higher temperatures has also been reported from inorganic precipitation experiments (Mucci, 1987; Oomori et al., 1987). However, the relative increase in Mg/Ca with temperature for foraminifera is greater than reported for inorganically precipitated calcites (~10% per °C compared to <5% per °C, respectively), suggesting that foraminiferal Mg/Ca is largely controlled by biological activity (Rosenthal et al., 1997; Erez, 2003; De Nooijer et al., 2009a, 2009b; Wit et al., 2012). The cellular control on Mg-incorporation is also reflected by the low Mg/Ca ratios of most foraminiferal species compared to calcite precipitated from seawater (Blackmon and Todd, 1959; Bentov and Erez, 2006). Furthermore, Mg/Ca varies also within single individual foraminifer tests (e.g. Hathorne et al., 2003; Toyofuku and Kitazato, 2005) and through chamber walls (e.g. Eggins et al., 2004; Sadekov et al., 2005; Kunioka et al., 2006; Hathorne et al., 2009).

The origin of intra-individual and intra-chamber wall Mg/Ca heterogeneity in benthic and planktonic species remains enigmatic. Previous work suggested that ontogenetic (i.e. life-stage related) changes can be responsible for intra- and inter-test Mg/Ca variability in benthic foraminifera (Hintz et al., 2006; Filipsson et al., 2010; Raitzsch et al., 2011a; Diz et al., 2012). Ontogeny can affect trace element partitioning through changes in physiology, growth rate or changes in surface area-volume ratios. Migration into different environments with age and life-stage may be responsible for part of the observed effects, but this is an environmental control rather than ontogeny as such. Additionally, inter-individual variability in Mg/Ca

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may potentially be caused by genetic differences between individuals of the same species (Numberger et al., 2009).

In a number of species, foraminiferal Sr/Ca is positively correlated with temperature (Rathburn and De Deckker, 1997; Reichart et al., 2003; Mortyn et al., 2005; Rosenthal et al., 2006; Kisakürek et al., 2011), salinity (Dissard et al., 2010a; Kisakürek et al., 2011) and seawater Sr/Ca (Delaney et al., 1985; Elderfield et al., 2000; Raitzsch et al., 2010). Reported inter- and intra-individual variability in Sr/Ca is smaller than observed for Mg/Ca (e.g. Dueñas-Bohórquez et al., 2009, 2011a). The partition coefficient for Sr incorporation is closer to that found in inorganic precipitation experiments (e.g. Lorens, 1981; Delaney et al., 1985; Tesoriero and Pankow, 1996; Dissard et al., 2010a) when compared to Mg incorporation (e.g. Delaney et al., 1985; Mucci, 1987; Nürnberg et al., 1996). This difference suggests that incorporation of Sr is not under the same biological control as Mg, and that the relatively weak control causes a more homogenous inter-species, intra-species and intra-individual Sr-incorporation.

The elemental and isotopic composition of individual tests is increasingly used for estimating past inter and intra-annual change, such as seasonality (Wit et al., 2010; Ganssen et al., 2011; Haarmann et al., 2011; Khider et al., 2011). The accuracy of such reconstructions, however, critically relies on the ability to distinguish vital effect-related variability from that caused by the environment. Here, we cultured genetically identical individuals of the shallow-water benthic foraminifer *Ammonia tepida* under constant environmental conditions, monitoring ontogenetic variability to constrain the intrinsic natural amplitude of Mg/Ca and Sr/Ca variability between and within individuals. *Ammonia* spp. produce calcite with a very low Mg/Ca (~1 mmol/mol), indicating a strong biological control on Mg-incorporation. This implies that changes in this biological control are also expected to have a relatively large impact compared to other foraminiferal species. Hence by studying this species we are able to capture the maximum impact related to variability in the vital effect. The intrinsic natural variability of Mg/Ca and Sr/Ca ratios ultimately determines how well we will be able to constrain past seasonal temperature variability and provides constraints for the biologically induced noise in reconstructions.

2. Methods

2.1. Culturing and reproduction

Surface sediments were collected from the muddy intertidal flats near Dorum, Northwestern Germany (53°44′16 N, 8°30′53 E) in Autumn 2008. Average Wadden Sea water temperature in October is ~13 °C, with an average diurnal variability of ~1 °C (Van Aken, 2008). Upon return to the laboratory, sediment was sieved over 1-mm screens to remove the largest macrofauna and stored at 10 °C. Prior to incubation, small amounts of sediment were sieved over a 250 μm-screen to retrieve large living individuals of *Ammonia tepida*. Individuals with brightly yellow colored cytoplasm were regarded as living and isolated for incubation. Four groups of 25 individuals were placed in Petri dishes with approximately 50 mL of 0.2 μm-filtered North Sea water (salinity 32), fed with living *Dunaliella salina* by adding 300 μL of a densely concentrated algae culture (approximately 3×10^6 cells/mL) and brought instantaneously to 25 ± 0.5 °C at which they were kept until reproduction.

Of all incubated specimens, 6 adult, megalospheric foraminifera underwent asexual reproduction at regular intervals, resulting in the production of 50–300 megalospheric, one-chambered juveniles emerging from the same adult test. Regular inspection of the foraminifera allowed recognition of reproduction when the juveniles were still in close proximity of their parent (Fig. 1). The size of these one-chambered juveniles ranges from approximately 20 to 30 μm, suggesting that they are gamonts (as opposed to schizonts; Goldstein and Moodley, 1993; Stouff et al., 1999). These juveniles were isolated from their parent test and kept together in a new Petri dish. All juveniles

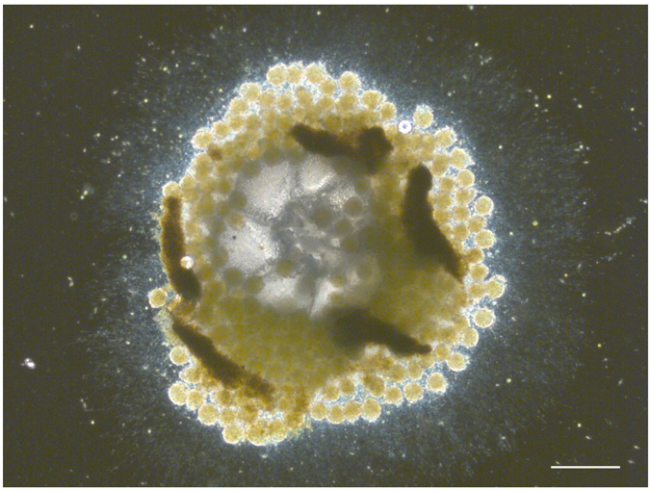

Fig. 1. Asexual reproduction in *Ammonia tepida*. The empty test of the adult (i.e. 'parent') individual is surrounded by approximately 200 juveniles that have emerged from the aperture. The single-chambered juveniles form a relatively large and dense pseudopodial network that they use to move away from the parent in the following hours. Clone groups were recognized and isolated when they were still in the vicinity of the parent test to prevent mixing of offspring from multiple reproductive events. The dark masses are remains of the food cyst that surrounded the adult foraminifer before reproduction. Scale bar = 100 μm.

resulting from one asexual reproductive event (i.e. having the same individual parent) will be referred to as a 'clone group'. Since the parents of these clone groups come from the same sample location, it may be that the different clone groups are genetically closely related. Juveniles from three of these clone groups were cultured in three separate Petri dishes and fed once a week. The water in these dishes was changed every two days. At the beginning and end of the incubations, salinity (32.2 ± 0.2) and pH (8.14 ± 0.05, NBS scale) were measured using a 330i WTW conductivity meter and WTW pH3000 with Schott BlueLine Electrodes, respectively. Culture media subsampled for DIC (2193 ± 29 μM) was filtered over 0.2 μm filters and measured photometrically using an XY-2 sampler (Bran + Lübbe GmbH, Norderstedt, Germany). Subsamples were also analyzed for $[Mg^{2+}]$ (49 ± 2 mmol/kg), $[Ca^{2+}]$ (9.5 ± 0.05 mmol/kg) and $[Sr^{2+}]$ (90 ± 0.8 μmol/kg) using ICP-OES. Uncertainties for these concentrations (±1 standard deviation) represent differences between replicate measurements (n = 4).

The three other clone groups were used to determine the growth rates by determining size of individuals by regular observation under an inverted microscope and counting the number of chambers of each individual. After 3 weeks of incubation in the same artificial seawater in which the other three clone groups were cultured, specimens were taken out and cleaned. Specimens both from the culturing experiment and from the incubation to determine growth rates were placed in buffered NaOCl (15%) for 24 h to remove organic material. Individuals were then rinsed several times with double deionized water and dried at 60 °C for several hours. Multiple individuals from one group with the same number of chambers (ranging from 6 to 16 chambers) were weighed on a UMX2 microbalance (Mettler Toledo, precision ± 0.1 μg) to determine "chamber-normalized" weights. Other studies have reported size-normalized weights based on the diameter (e.g. De Moel et al., 2009; Beer et al., 2010) of analyzed individuals, but to facilitate comparison of growth with the LA-ICP-MS data (see Section 3.3) size-normalized weights and growth rates are expressed per total number of chambers.

Since a large number of geno- and morphotypes have been reported for *Ammonia* (e.g. Holzmann and Pawlowski, 1997; Debenay et al., 1998; Holzmann and Pawlowski, 2000; Hayward et al., 2004), culturing individuals from one clone group overcomes the potential imprint of genetic variability on calcite chemistry. This is particularly important since the genus of *Ammonia* is widely studied, but named differently