Chamber formation leads to Mg/Ca banding in the planktonic foraminifer *Neogloboquadrina pachyderma*

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Highlights:

- Large amplitude Mg/Ca variability (<0.1-7 mmol/mol) at constant temperatures
- High Mg/Ca bands deposited on outside of laminae at the end of chamber formation
- Crust Mg/Ca similar to laminar calcite and biologically controlled

24   •   Temperature influence on Mg/Ca despite large intra- and interest variability

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Abstract

Many species of planktonic foraminifera show distinct banding in the intratest distribution of Mg/Ca. This heterogeneity appears biologically controlled and thus poses a challenge to Mg/Ca paleothermometry. The cause of this banding and its relation with chamber formation are poorly constrained and most of what we know about intratest Mg/Ca variability stems from culture studies of tropical, symbiont-bearing foraminifera. Here we present data on the non-spinose, symbiont-barren *Neogloboquadrina pachyderma* from the subpolar North Atlantic where wintertime mixing removes vertical gradients in temperature and salinity. This allows investigation of biologically controlled Mg/Ca intratest variability under natural conditions. We find that intratest Mg/Ca varies between <0.1 and 7 mmol/mol, even in winter specimens. High Mg/Ca bands occur at the outer edge of the laminae, indicating reduced Mg removal at the end of chamber formation. Our data thus provide new constraints on the timing of the formation of such bands and indicates that their presence is intrinsic to the chamber formation process.

Additionally, all specimens are covered with an outer crust consisting of large euhedral crystals. The composition of the crust is similar to the low Mg/Ca bands in the laminar calcite in winter and summer specimens, indicating a tight biological control on crust formation and composition. Nevertheless, despite high intratest variability, the median Mg/Ca of summertime tests is higher than that of wintertime tests. This provides support for Mg/Ca paleothermometry, but to improve the accuracy of paleotemperature estimates biological effects on Mg incorporation need to be better accounted for.

1. Introduction

The Mg/Ca ratio in foraminiferal calcite is widely used to reconstruct seawater temperatures (Barker et al., 2005; Jonkers et al., 2010b; Lea et al., 2000; Mashiotta et al., 1999). However, Mg/Ca-temperature relationships are species specific and remain (largely) empirical and many studies have shown a tight biological control on Mg incorporation in foraminiferal calcite (Anand et al., 2003; Bentov and Erez, 2006; de Nooijer et al., 2014; Jonkers et al., 2012; Jonkers et al., 2013a; Lea et al., 1999; Spero et al., 2015). In particular, the Mg distribution within single foraminifera tests shows large, non-temperature related variability (Eggins et al., 2003; Eggins et al., 2004; Kunioka et al., 2006; Sadekov et al., 2005). Accurate paleotemperature reconstructions thus require a solid understanding of the nature of and controls on such variability.

Magnesium concentrations in foraminiferal calcite are orders of magnitude lower than in seawater and thus indicate active discrimination against the incorporation of Mg during calcification (e.g. Bentov and Erez, 2006; de Nooijer et al., 2014). Planktonic foraminifera build their skeleton by progressively adding chambers. In the ideal case, each time a new chamber is added the older chambers are covered by an additional layer of calcite (Erez, 2003; Hemleben et al., 1977; Reiss, 1957), resulting in a laminated structure. Mg/Ca (and other trace element) banding has been observed in this laminar calcite (Eggins et al., 2004; Erez, 2003; Hathorne et al., 2009; Kunioka et al., 2006; Sadekov et al., 2005). Yet, this intratest Mg/Ca heterogeneity is too large to be caused by temperature variability experienced during the growth of the foraminiferal test and Eggins et al. (2004) attributed such banding in the final chamber of *Orbulina universa* to pH changes as a result of diurnal changes in endosymbiont activity. However, similar banding was later also observed in symbiont-barren species, indicating that other mechanisms are required (Hathorne et al., 2009). Some researchers have suggested that Mg-banding results from the presence of organic layers in the calcite lattice (Kunioka et al., 2006), but in this study both the number of high Mg/Ca bands as well as their position do not appear to match with the organic layers. Others have also shown that high Mg/Ca bands do not occur at the location of organic layers, rendering this explanation for intratest trace element banding unlikely (Eggins et al., 2004; Sadekov et al., 2005; Spero et al., 2015). Alternative hypotheses involve the participation of a tiny amount of seawater (which has high Mg

75 concentrations) at the initiation of chamber formation, which would result in a high Mg band at the

76 beginning (inner edge) of each lamina (de Nooijer et al., 2014), or an active role for mitochondria in

77 Mg removal from the site of calcification, such that their reduced activity or density, possibly diurnally

78 modulated, may cause high Mg bands (Spero et al., 2015). Nevertheless, the origin of Mg-banding is

79 not yet fully understood and the suggested mechanisms are to a large extent based on observations

80 from a single tropical and symbiont-bearing species *O. universa*, which forms a unique spherical outer

81 chamber and hence differs from other species of planktonic foraminifera (Spero, 1988). To distinguish

82 between these latter two hypotheses it is necessary to determine whether the high Mg/Ca bands are

83 formed at the start of chamber formation or not. As such it is important to determine the intratest

84 Mg/Ca distribution of planktonic foraminifera that undergo a typical lamellar calcification process.

85 Various genera of planktonic foraminifera also form an outer crust at the end of their life cycle (Bé et

86 al., 1979; Hemleben et al., 1977). In some species crust formation is related to gametogenesis (Bé,

87 1980). However in many Globorotaliids and Neogloboquadrinids the relationship with gametogenesis

88 is less clear and it is thought that crusts are formed deep in the water column possibly in response to

89 a temperature trigger (Hemleben and Spindler, 1983; Kohfeld et al., 1996; Srinivasan and Kennett,

90 1974). These outer crusts consist of large euhedral calcite crystals covering the laminar calcite and

91 their function remains poorly understood. Importantly however, encrustation also presents another

92 source of intratest Mg/Ca variability since crusts often have lower Mg/Ca ratios than the interior

93 lamellar calcite (Bolton and Marr, 2013; Eggins et al., 2003; Fehrenbacher and Martin, 2010; Jonkers

94 et al., 2012; Sadekov et al., 2005). This appears to be consistent with crust formation occurring at

95 greater depths in the water column and it has consequently been suggested that single crust-bearing

96 tests could (at least theoretically) be used to reconstruct vertical water column temperature profiles

97 (Eggins et al., 2003; Sadekov et al., 2009). However, encrustation is variable both in thickness and

98 composition (Jonkers et al., 2012; Steinhardt et al., 2015) and the use of single foraminifera as past

99 tracers of water column properties relies on an improved understanding of the factors controlling

100 crust formation.

101 To assess the causes and consequences of Mg/Ca banding and encrustation we investigate Mg/Ca

102 distribution within *Neogloboquadrina pachyderma* tests at high resolution. *N. pachyderma* is a non-

103 symbiotic species that shows normal lamellar calcification and is often used in paleoceanographic

104   reconstructions. The species dominates high latitude assemblages and could hence be an excellent

105   species to serve as a cold-end paleothermometer, but the use of its Mg/Ca is confounded by

106   encrustation and/or non-temperature related effects (Jonkers et al., 2013a; Meland et al., 2006). We

107   use samples from a sediment trap moored in the subpolar North Atlantic Ocean (59.3° N, 39.7° W,

108   2750 m water depth) and exploit the unique conditions at the site to assess intratest Mg/Ca

109   heterogeneity in the absence of temperature/environmental variability.

110   2. Oceanographic setting

111   The *in situ* temperature and salinity data from Central Irminger Sea mooring (59.7° N, 39.7° W; 45 km

112   north of the sediment trap) shows a pronounced seasonal cycle in near surface temperature that

113   quickly attenuates with depth (Fig. 1). At 10 m depth seawater temperatures vary from approximately

114   10 °C in early autumn to around 4.5 °C between January and May, when they reach values similar to

115   those found at greater depths. Consequently, there is no, or only a negligible, vertical thermal

116   gradient (i.e. isothermal conditions) in winter and spring (Fig. 1). Near surface salinity variability is less

117   than 0.4 and similar to temperature, the vertical salinity gradient disappears from January onwards

118   (Fig. 1). Moreover, wintertime convective mixing down to at least 400 m water depth most probably

119   eliminates vertical gradients in any other environmental parameters (de Jong et al., 2012).

120   Foraminifera living during the winter period have thus not experienced any significant temperature,

121   salinity or other environmental variability, making this period/site a natural laboratory to study

122   biological effects on test geochemistry.

123   3. Material and methods

124   Tests of *N. pachyderma* were picked from the 150-250 μm fraction of three sediment trap samples

125   with 16-day collection intervals. These represent one sample from the stratified warm season flux

126   pulse (5 to 20 September 2006; IRM4 A-01) and two samples from the isothermal cold season at the

127   start of the spring bloom (1 to 16 April and 17 April to 2 May 2007; IRM4 A-14 and IRM4 A-15). Two

128   tests were analysed from each sample. Taking the settling time and life span of planktonic

129   foraminifera into account, the foraminifera from these samples most likely formed their tests up to

130   approximately a month before the start of the collection interval. Water column abundances of *N.*

131   *pachyderma* in the Nordic and Labrador Seas are highest in the upper 200 m (Carstens et al., 1997;

132   Kohfeld et al., 1996; Stangeew, 2001) and most calcification is likely to take place in this zone (Jonkers

133   et al., 2010a). Specimens from the warm season samples could thus have experienced up to ~5 °C

134   temperature variability, whereas those from spring were formed in very stable conditions with

135   changes in temperature ≤0.5 °C (Fig. 1).

136   For an extended description of the sediment trap mooring and sample treatment see Jonkers et al.

137   (2010a; 2013a; 2013b). All tests were cleaned using repeated brief ultrasonication in deionized water

138   and methanol prior to embedding in Araldite 20/20 resin. Samples were left to harden at room

139   temperature for at least 24 hrs and polished using fine sandpaper and a 0.3 μm Al_2O_3 emulsion.

140   Scanning electron microscope (SEM) images were taken before and after embedding.

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142   Element concentrations were measured using quantitative X-ray mapping on a JEOL 8530F field-

143   emission electron microprobe equipped with 5 wavelength dispersive spectrometers (WDS) at the

144   University of Bristol. The maps were acquired at 15 kV and 40 nA with a pixel size of 0.3 μm. To

145   ensure the stability of the sample and achieve meaningful counting statistics the samples were coated

146   with silver (Kearns et al., 2014; Smith, 1986). Ca was measured first for 10 ms on two spectrometers

147   (a PETH and PETL crystal), Mg was measured subsequently for 300 ms on three spectrometers (2 TAP

148   crystals and a TAPH crystal). Diopside was used as a standard for both Mg and Ca. High Mg/Ca bands

149   of 1-2 pixel width are clearly identifiable (Fig. 2-4), suggesting the resolution to be on the order of 0.3-

150   0.6 μm. However, X-rays are emitted from a larger area with the analytical resolution approximating

151   0.9 μm (calculated using PENEPMA (Salvat et al., 2006) and defined as the distance over-which 75% of

152   x-rays are emitted). This means that for any band <0.9 μm the intensity will be a convolution of the

153   band measured and the adjacent material. The detection limit for Mg for an average of 4 pixels is 120

154   ppm and analytical uncertainty at Mg/Ca ratios ~4 mmol/mol amounts to ~11 % RSD. To obtain a

155   comparable precision at lower Mg/Ca ratios more pixels counts need to be integrated. For Mg/Ca

156   ratios around 1 mmol/mol 80 pixels (7.2 μm^2; approximately ¾ of the scale bar in Fig. 2-4) are

157   required. Integrating over an area this size reduces the limit of detection to below 20 ppm and still

158   allows the accurate determination of the low Mg/Ca ratios of the crust and lamellar calcite since

159   these are relatively homogenous. The maps were quantified using CalcImage (part of the Probe for

160   EPMA software – ProbeSoftware Inc.) in which the full Armstrong-Love/Scott matrix correction

161   (Armstrong, 1988) is applied to each pixel. To generate the Mg/Ca maps, pixels with Ca wt.% below 35

162   were masked and negative Mg values were substituted with half of the minimum value above zero.

163   For the map in Fig. 4E no reliable Ca data were available due to beam damage of the test surface and

164   in this case a Ca content of 40 wt.% is assumed, in line with the calcite composition of the tests.

4. Results

166   All tests used in this study were encrusted, showing large euhedral crystals on the outside of the test

167   surface (Fig. 2-4). Encrustation is not always homogeneous across the test and the older chambers are

168   often covered with a denser crust consisting of larger crystals. Tests from the cold season have

169   generally lower Mg/Ca than those from the warm season (median values: 0.9 and 2.1 mmol/mol,

170   respectively) and all tests, including those from the cold season, have highly variable Mg/Ca ratios,

171   ranging between <0.1 and >7 mmol/mol (Fig. 2-5). In general, Mg/Ca ratios in the chambers of the

172   initial whorl appear to be higher than in the subsequent chambers (although this might in part be due

173   to relief; Fig. 3E; 4F and 4G).

174   All tests show thin (1-2 μm) high Mg/Ca bands within the lamellar calcite (Fig. 2-4; it should be noted

175   that the very high Mg/Ca bands at the outside of the tests must be considered with caution as these

176   may be due to edge effects). The distribution of Ca in the tests is homogeneous and does not show

177   any sign of the banding that is present in the Mg/Ca (supl. fig). Older chambers often show multiple

178   high Mg/Ca bands, with variable Mg/Ca ratios (~2-7 mmol/mol; Fig. 2-4). Importantly, all high Mg/Ca

179   bands occur at the outer edge of the laminae, which can most clearly be seen in test 15_2 where the

180   band extends along the outer edge of the laminar calcite and is covered by a crust on the outside of

181   the test (Fig. 2G and 2I, and also in 15_1 (Fig. 2E and 2F) and 14_2 (Fig. 3I)). We observe no consistent

182   difference in the Mg/Ca ratio of the bands between the warm and cold season.

183   The outer crust is relatively homogeneous and compositionally similar, if not identical to the low

184   Mg/Ca bands of the lamellar calcite (Fig. 2-4). This pattern is clearest in tests from the cold season,

185 but is also present in specimens from the summer season, where in some cases the Mg/Ca ratio of

186 the crust is even higher than that of the lamellar calcite (Fig. 4 and 6). Two specimens (14_2 and 01_2)

187 suggest layering within the crust, as thin bands of higher Mg/Ca are present at the very outer edge of

188 the test (Fig. 3I and 4H).

5. Discussion

190 The data presented here demonstrate a more complex distribution of Mg/Ca in the tests of

191 Neogloboquadrinids than found in previous studies, which showed a simple two-layered structure

192 with a high Mg/Ca inner layer and a low Mg/Ca crust on the outside of the tests (Eggins et al., 2003;

193 Jonkers et al., 2012; Sadekov et al., 2005; Steinhardt et al., 2015). These studies attributed this simple

194 layering to encrustation taking place at greater depths due to downward migration during ontogeny.

195 Only Fehrenbacher and Martin (2010) reported the presence of complex layering similar to our

196 observations in *N. dutertrei*, but they did not discuss its origin.

197 Assuming the generally accepted Mg/Ca temperature sensitivity of ~10%/°C (Anand et al., 2003;

198 Elderfield and Ganssen, 2000) the intratest range in Mg/Ca we observe in both warm and cold season

199 tests would translate to a temperature range of >40 °C, which is clearly unrealistic. The presence of

200 large amplitude Mg/Ca banding in cold season tests that experienced negligible temperature

201 variability also rules out that the banding directly results from vertical water column migration even if

202 the sensitivity would be higher. Given the generally short (~ 1 month) life span of planktonic

203 foraminifera (Hemleben et al., 1989; Jonkers et al., 2015; Volkmann, 2000), temporal seawater

204 temperature variability can also be discounted as a driver of the intratest Mg/Ca heterogeneity. In

205 addition, the cold season specimens are highly unlikely to be derived from surviving remnants of the

206 previous summer population as we observe that the test flux starts to increase during the collecting

207 interval (Fig. 1) and stable oxygen and carbon isotopes measured in co-collected specimens suggest

208 typical cold-water conditions (Jonkers et al., 2010a; Jonkers et al., 2013b). These tests thus stem from

209 the new (spring bloom) population and given the constant environmental conditions in the water

210 column experienced during their growth, we conclude that the intratest Mg/Ca variability reflects a

dominant biological control on Mg incorporation and is not the result of external variability in

temperature, salinity or other environmental parameters.

The banding in the tests of *N. pachyderma* is only observed in the Mg/Ca and not in the Ca maps,

indicating that the high Mg bands are not associated with organic layers within the calcite lattice since

these would show as layers of reduced Ca intensity (Sadekov et al., 2005). Moreover, the presence of

high Mg/Ca bands in the laminae covering preceding chambers (Fig. 2-4) rules out an association with

the primary organic membrane (POM) since this membrane is only present in the newly formed

chamber and not in the lamina covering the older chambers. Similarly, it has been shown that in *O.*

universa the POM occurs in a broad band of low Mg/Ca (Eggins et al., 2004; Spero et al., 2015) and

Sadekov et al. (2005) showed that high Mg/Ca bands in *Globorotalia truncatulinoides* are not

associated with embedded organic layers. We can therefore reasonably conclude that the Mg/Ca

banding in *N. pachyderma* is also unlikely to be result of organic layering in the test wall. As such,

consistent with most recent hypotheses on Mg/Ca banding (de Nooijer et al., 2014; Spero et al.,

2015), we propose that such banding is intrinsic to the calcification mechanism of planktonic

foraminifera. Below we discuss Mg/Ca heterogeneity in the lamellar and crust calcite, as well as the

paleoceanographic implications of this intratest compositional variability.

5.1. Mg/Ca banding in lamellar calcite

In *N. pachyderma* each lamina consists of a low Mg/Ca band of variable width (max. 10 µm) on the

inside and a thin (1-2 µm) high Mg/Ca band on the outside. This pattern is visible in most tests and

older chambers, in general, show (approximately) the number of bands expected from laminar

growth of the test. Some chambers show fewer bands, which may result from absence of the laminae

since calcification does not always strictly follow a laminar pattern, i.e. the layer from subsequent

chamber does not always cover all preceding chambers and becomes thinner towards older chambers

(Hemleben et al., 1977; Nehrke et al., 2013). In addition, the laminae may be too thin to be resolved

using our techniques and/or merge with the previous lamina (Fig. 2E). Alternatively, since the

composition of the high Mg/Ca bands varies, the contrast between the low and high Mg/Ca bands

may be too small to allow distinction (see for instance 15_2, where the band is faintly visible in Fig 2H,

but not in 2G even though they depict the same chamber). One or more laminae may thus be missing

239   from individual chambers, explaining the occasional absence of multiple bands in older chambers.

240   Thus, we feel confident to suggest that typically each lamina ends with the deposition of a high Mg/Ca

241   calcite band, which strongly suggests that discrimination against Mg incorporation into the calcite

242   diminishes towards end of chamber formation.

243   As such our observations add important new constraints on the origin of the banding as they

244   constrain the timing of its formation within the chamber formation process. Our results rule out the

245   recent suggestion of the participation of a tiny amount of unaltered seawater with high Mg

246   concentrations at the initiation of chamber formation (de Nooijer et al., 2014) and instead suggest

247   that foraminifera are able to effectively control the composition of the calcifying fluid from the onset

248   of chamber formation. This may point to the existence of an internal cation pool that becomes

249   depleted in Ca during calcification, leaving a solution behind that is enriched in Mg, which is used at

250   the final stage of chamber formation (Bentov et al., 2009; Erez, 2003). However, this process cannot

251   reflect simple Rayleigh fractionation, as we do not observe a gradual increase in Mg/Ca from the

252   inside to the outside of the lamina. Hathorne et al. (2009) have also shown that such a mechanism

253   cannot account for trace element banding in other species. Moreover, the very existence of an

254   internal cation pool has recently been contested (de Nooijer et al., 2014; Mewes et al., 2015; Nehrke

255   et al., 2013), rendering this explanation unlikely.

256   Recent studies on *O. universa* have shown that the Mg/Ca banding within the final chamber reflects a

257   diurnal rhythm, with the precipitation of a high Mg/Ca band at night (Eggins et al., 2004; Spero et al.,

258   2015). Since *N. pachyderma* does not possess endosymbionts, it is clear that the banding in this

259   species cannot result from pH changes as a result of varying symbiont activity (Eggins et al., 2004).

260   While it is possible that the thin high Mg/Ca bands represent short episodes of rapid calcification,

261   others have shown that changes in the calcification rate are unlikely to be the cause of banding in

262   foraminiferal tests (Hathorne et al., 2009).

263   Our data show for the first time that each lamina ends with a high Mg/Ca band, suggesting that the

264   banding is an integral part of the chamber formation process. It may be that the chamber formation

265   in *N. pachyderma* is comparable to the wall thickening of the final chamber in *O. universa* and that the

266   banding results from changes in the effectiveness of Mg removal from the location of calcification.

267   Recent work suggested that the efficiency of Mg removal in *O. universa* could be regulated by

268   changes in the activity and/or density of mitochondria (Spero et al., 2015) and a similar mechanism

269   may play a role in *N. pachyderma*. However, little is known about chamber formation in this species

270   and there is no *a priori* reason to assume that chamber formation and/or mitochondrial activity and

271   hence the banding is diurnally paced. Thus, while variable Mg removal by mitochondria presents a

272   likely mechanism for the banding in *N. pachyderma*, the reduction in Mg removal may simply be

273   intrinsic to the chamber formation process, with the mitochondrial activity related to the organism's

274   function and energy balance.

275   5.2. Mg/Ca ratios of the crust calcite

276   All specimens investigated here are encrusted and the presence of crusts in cold season specimens,

277   when the water column is fully mixed, argues against temperature trigger of crust formation as

278   suggested for *N. dutertrei* (Hemleben and Spindler, 1983). The crusts appear thicker on the older

279   chambers in the final whorl (Fig. 2-4). This has been observed before (Srinivasan and Kennett, 1974)

280   and in *N. dutertrei* the thinner crust on the younger chambers was found to have lower Mg/Ca ratios,

281   which was taken as evidence for gradual crust formation during late ontogeny (Jonkers et al., 2012;

282   Steinhardt et al., 2015). Higher Mg/Ca ratios in the crust covering the youngest chambers than in the

283   older chambers are not clearly evident in our new data, but the warm season specimens show

284   enhanced variability in the Mg/Ca ratio of the crust. This is consistent with the suggestion of a tight

285   biological control on crust formation and composition (Jonkers et al., 2012; Steinhardt et al., 2015).

286   Taken together with the layering observed in the crust of specimens 14_2 and 1_2 these data

287   highlight the need for additional studies to constrain the processes governing crust formation.

288   Cold season specimens show no compositional difference between the Mg/Ca ratio of the crust and

289   the low Mg/Ca lamellar calcite in (Fig. 2 and 3). This could be expected because the water column was

290   well mixed and isothermal. However, neither during the warm season do the crusts have consistently

291   lower Mg/Ca ratios than the lamellar calcite and in fact the opposite occurs in tests from sample 01

292   (Fig. 4 and 6). There is thus no clear indication that the crust is formed at greater depth, either

293   because the foraminifera are able to control their Mg/Ca ratio independent of temperature, or

294   because they remain at a relatively shallow depth throughout their life cycle. The latter would be

295   consistent with the large amplitude in the stable isotope composition of samples from the same time

series (Jonkers et al., 2010a; Jonkers et al., 2013b) and with observations of encrusted tests at

relatively shallow depths (Bauch et al., 1997; Kohfeld et al., 1996). Either way, our new data caution

against the use of Mg/Ca in single specimens of *N. pachyderma* as a simple water column

temperature profiler.

5.3. Implications for Mg/Ca paleothermometry

Many studies have shown a clear relationship between Mg/Ca in foraminiferal calcite and calcification

temperature (Anand et al., 2003; Elderfield and Ganssen, 2000; Nürnberg, 1995). And even though

intertest variability within single species of foraminifera is large, when multiple individual tests are

analysed, similar temperature relationships are observed on a population level (Haarmann et al.,

2011; Marr et al., 2011; Sadekov et al., 2009). In addition, despite large variability within tests, Spero

et al. (2015) observed that the Mg/Ca ratio of both the low and high Mg/Ca bands in *O. universa*

increased with temperature. This again suggests that on a population level Mg/Ca and calcification

temperatures are correlated, but that at the level of individual tests, biological controls may overprint

the temperature signal.

Previously measured bulk solution-based Mg/Ca data from the same time series are only available for

samples IRM4 A-01 and A-15 and are 1.35 and 1.21 mmol/mol, respectively, thus falling in the range

of our new observations (Jonkers et al., 2013a). While these bulk Mg/Ca analyses revealed little

temperature dependence of Mg/Ca, the new observations on a small number of tests suggest that

warm season tests have higher Mg/Ca ratios than cold season tests (Fig. 5). However, other studies

have shown large intertest Mg/Ca variability in planktonic foraminifera (e.g. Haarmann et al., 2011;

Jonkers et al., 2012) and we cannot rule out that our new data on a limited number of tests fall within

the range of natural variability. Nevertheless, the difference between tests grown in warm and cold

water is solely due to higher Mg/Ca in the low Mg/Ca lamellar calcite and in the crust and we do not

observe any consistent difference in the high Mg/Ca bands, such as observed in *O. universa* (Spero et

al., 2015). Consequently, biological imprints on paleotemperature estimates would depend on the

proportion of the high Mg/Ca bands with respect to the entire test volume as well as the contrast

between the low and high Mg/Ca layers. In the tests analysed here, calcite with >4 mmol/mol Mg/Ca

makes up <4% of the total surface of the cross section (except in 01_2). Assuming that this proportion

is representative of the entire test, it is evident that even a small contribution of high Mg/Ca calcite

may increase the estimated calcification temperature, particularly at low temperatures where the

slope of the calibration curve is lower. For example, given modal Mg/Ca values around 0.5-0.6

mmol/mol (comparable to test 15_1; Fig. 2), the presence of bands with an Mg/Ca ratio ~6 mmol/mol

making up only 2% of the volume would increase the overall Mg/Ca ratio by ~15 %. With a

temperature sensitivity of ~10%/°C this would lead to an increase in the inferred calcification

temperature that is significantly larger than the combined analytical and calibration error of 1°C

(Anand et al., 2003). It is therefore clear that biologically controlled changes in the Mg/Ca ratio of

foraminifera can significantly affect paleotemperature estimates. At the same time, the apparent

positive relation between modal Mg/Ca and temperature provides support for the use of Mg/Ca to

reconstruct seawater temperatures. This dichotomy highlights one of most intriguing outstanding

questions surrounding the use of trace element based foraminiferal proxies: why does the test

geochemistry on a population level appear to respond to environmental conditions/stimuli, whereas

inter- and intra-test variability appear (orders of magnitude) larger and virtually exclusively

biologically controlled?

6. Conclusions

Wintertime vertical mixing of the water column in the central Irminger Sea renders vertical gradients

in temperature, salinity and other environmental parameters negligible, making this site ideal to

study planktonic foraminiferal chemistry in the natural environment. Electron microprobe analyses of

N. pachyderma tests from a sediment trap time series reveal that:

- Mg/Ca ratios in tests of *N. pachyderma* vary between <0.1 and 7 mmol/mol in the absence of

  temperature or other variability. The lamellar calcite consists of layers of low Mg/Ca inner

  and high Mg/Ca outer bands. Occasional banding occurs in the crust that covers the outer

  chambers of each test.

- The presence of high Mg/Ca bands on the outside of each lamina indicates a decrease in

  discrimination against Mg incorporation into the calcite towards the end of chamber

  formation.

351　　• The Mg/Ca banding appears intrinsic to the chamber formation process. This is consistent

352　　　with a recent study (Spero et al., 2015) that surmises a role for mitochondria in the

353　　　(variable) removal of Mg from the location of calcification. However, the (physiological)

354　　　reason for variable mitochondrial activity remains to be established and need not necessarily

355　　　be diurnally paced as in *O. universa*.

356　　• All tests analysed in this study had a rough crystalline crust on the outside of the tests. The

357　　　presence of such a crust in specimens that only experienced isothermal conditions precludes

358　　　a thermal trigger for encrustation and indicates a tight biological control for crust formation.

359　　• The Mg/Ca ratios of the crust are similar or higher than the low Mg/Ca bands in the laminar

360　　　calcite. This suggests that its composition is biologically controlled and/or that the crust is

361　　　formed relatively shallow in the water column. These observations argue against the use of

362　　　single tests as paleotemperature profilers.

363　　• In general tests from the warm season show higher Mg/Ca than those from the cold season,

364　　　supporting Mg/Ca paleothermometry. However, large inter- and intratest Mg/Ca variability

365　　　appears biologically controlled and the processes governing this variability need to be better

366　　　constrained to improve the accuracy of past seawater temperature estimates.

Acknowledgements

Figure captions

Fig. 1: temperature, salinity and *N. pachyderma* test flux (150-250 μm) in the central Irminger Sea between September 2006 and mid May 2007. The red dot in the map inset shows the locations of the moorings in the Irminger Sea southeast of Greenland. The red line in the temperature graph highlights the winter and spring period when the vertical thermal gradient ≤ 0.5 °C. The horizontal black bars at the bottom indicate the collection intervals of the samples used, with the stippled lines indicating the possible interval when the foraminifera in these samples could have been living. Specimens from IRM4 A-14 and A-15 thus experienced negligible temperature and salinity variability during their life cycle.

Fig. 2: SEM images of *N. pachyderma* tests from sample IRM4 A-15 (cold season) before and after cross sectioning as well as Mg/Ca (mmol/mol) maps of the areas indicated by the orange rectangles in the upper panels. Note the high Mg/Ca bands at the end each lamina and the absence of a compositional difference between the crust and the low Mg/Ca bands of the laminar calcite.

Fig. 3: SEM images and Mg/Ca (mmol/mol) maps for sample IRM4 A-14 (cold season). Note the apparent layering in the crust in I and J.

Fig. 4: SEM images and Mg/Ca (mmol/mol) maps for sample IRM4 A-01 (warm season). Note the lower Mg/Ca ratio in the lamellar calcite than in the crust in F (upper chamber; F-1) and G (upper chamber; F-0). The grey lines in F and G show the approximate position of the profiles shown in figure 6.

Fig. 5: Lower Mg/Ca in cold season *N. pachyderma* tests: histograms of Mg/Ca ratios from the warm and cold season. Median Mg/Ca values are indicated by the black dots.

Fig. 6: Mg/Ca profiles across the summer season tests. The location of the profiles is indicated by the grey lines in figure 4F and G; roman numbers define start and end points of the paths (Fig. 4). Note

404   that the Mg/Ca ratio of the crust – highlighted in grey – is higher or indistinguishable from the low

405   Mg/Ca lamellar calcite. Errors are based on a profile width of 12 μm.

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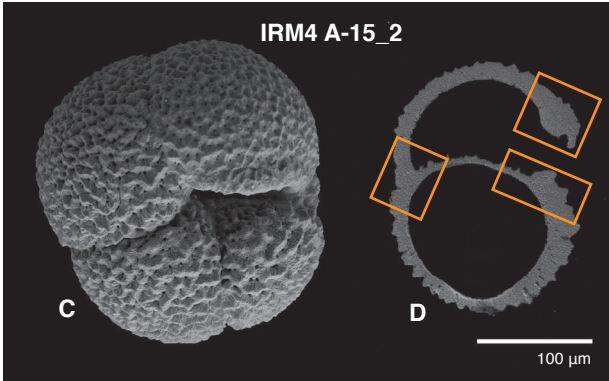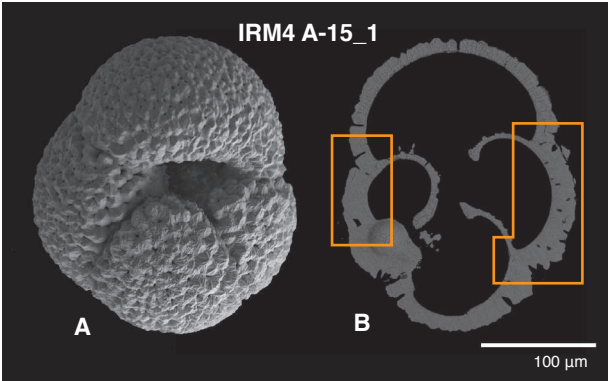533   Albrechts-Universität zu Kiel, 98 pp.

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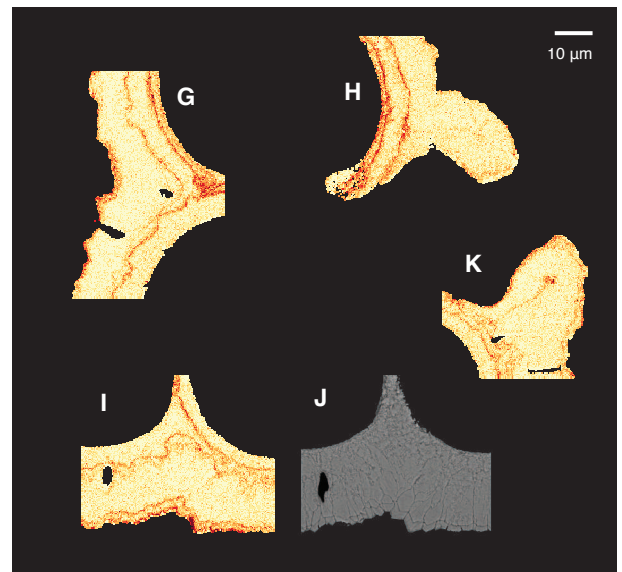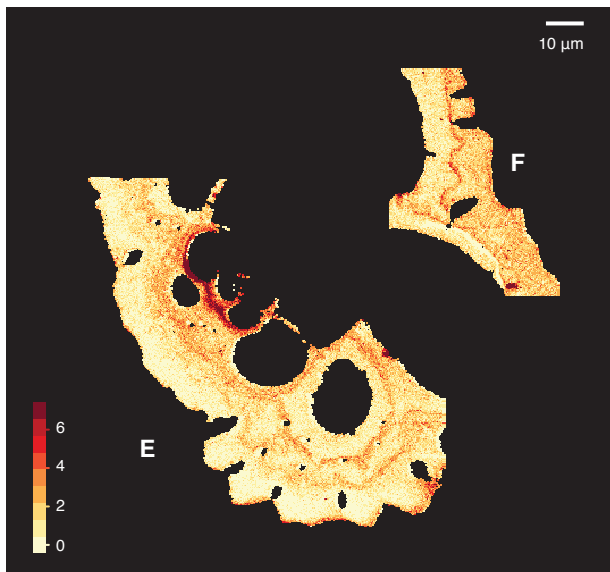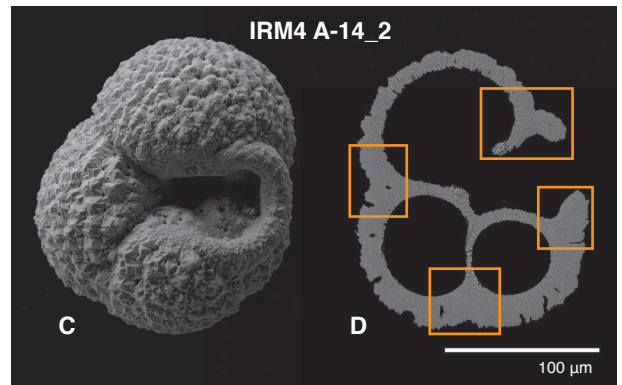537   distribution and ecology. The Journal of Foraminiferal Research 30, 157-176.

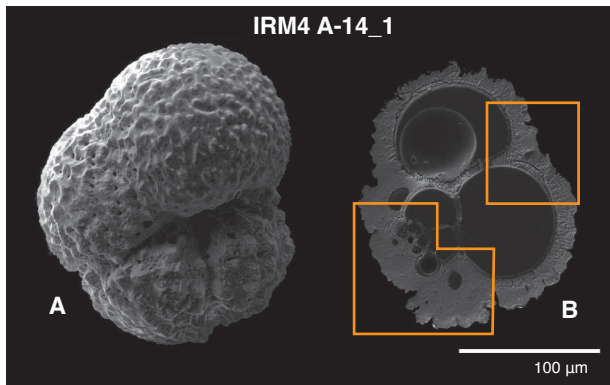538

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