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Title:
Environmental impacts on foraminiferal calcification during the Pliocene

Environmental impacts on foraminiferal calcification

during the Pliocene

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Masters of Palaeobiology

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Key Points:

- Pliocene planktic foraminiferal size decreases as a response to increased ocean stratification

- Size-normalised weight of *Globeriginoides ruber* (white) decreases during glacials, challenging the view of oceanic partial pressure of carbon dioxide as a driver for calcification

- Amplitudes of climate change impact planktic foraminiferal calcification; there is greater decline of calcification during events of larger, and more rapid, rising pCO$_2$

Abstract:

Anthropogenic atmospheric carbon dioxide (pCO_2^{atm}) is rapidly rising. This increase is impacting on the ocean, affecting ocean chemistry, the biological pump, and marine organisms. Marine calcifiers are predicted to be affected by increased CO_2, but future consequences are still unknown. To assess potential impacts, this project established the relationship between changing environmental conditions and calcification in foraminifera, a group essential to the carbon cycle. The Pliocene was the last time interval when pCO_2^{atm} concentrations were comparable to today. Test size and weight of the dominant species, *Globigerinoides ruber* (white), was quantified through the Pliocene at Ocean Drilling Program Site 999 in the Caribbean to assess impacts on carbonate production. Test size shows high-frequency variability not related to carbonate chemistry or temperature. During the cold interval marine isotope stage (MIS) M2, foraminiferal test size increased potentially linked to intensified stratification in response to the closure of the Central American Seaway (CAS). The foraminiferal size-normalised weight (SNW) of *G. ruber* decreases during MIS M2 and is heavier at higher temperatures and thereby for inference higher pCO_2^{atm}; challenging the view of oceanic partial pressure of carbon dioxide (pCO_2) as a simple driver for calcification. To better understand the drivers of changes in SNW, CT scans were used to calculate calcite to volume (CV) ratios and test thickness in the penultimate chamber. This data suggests the driver of weight change in foraminifera is unlikely due to increased pCO_2, but rather biotic factors; despite reduced CV ratios during warmer interglacial periods.

Keywords:

Pliocene, planktic foraminifera, calcification, climate change, carbon dioxide

Declaration

I declare that the work in this thesis was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Taught Postgraduate Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, this work is the author's own, informed by discussions with the supervisor. Work done in collaboration with, or with the assistance of others, is indicated as such. I have identified all material in this dissertation which is not my own work through appropriate referencing and acknowledgement. Where I have quoted from the work of others, I have included the source in the references/ bibliography. Any views expressed in the dissertation are those of the author and do not represent those of the University of Bristol.

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Chloe Louise Todd   Date

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1. Introduction

1.1. Implications of anthropogenic climate change

Atmospheric carbon dioxide (pCO$_2$^{atm}) has increased significantly from 280 parts per million (ppm) at the start of the industrial revolution (1750) [Siegenthaler et al., 2005] to 407.6 ppm today (2017) [NASA, 2017]. It is predicted to rise to between 530 to 720 ppm by 2100 under the Intergovernmental Panel on Climate Change (IPCC) RCP4.5 intermediate emissions scenario [Pachauri et al., 2014]. 30 % of this anthropogenic CO$_2$ is being absorbed by the ocean [Feely et al., 2004; Sabine and Feely, 2007; Sabine et al., 2004] causing an increase in oceanic partial pressure of CO$_2$ (pCO$_2$). In response, proton concentrations in the ocean have increased by 30 %, with projections suggesting that surface pH will drop by 0.20 to 0.21 units (58 to 62%) by the end of the 21st century, relative to pre-industrial values [Pachauri et al., 2014].

Although the ocean is absorbing vast quantities of CO$_2$, there is a concern it is becoming increasingly limited in its capacity as a carbon sink [Sabine et al., 2004; Thomas et al., 2008]. Moreover, the absorption of CO$_2$ by the ocean reduces the saturation state of calcite and aragonite and, as a result, both saturation horizons are caused to shoal, increasing dissolution of calcium carbonate (CaCO$_3$) [Fabry et al., 2008; Feely et al., 2004; Ridgwell and Schmidt, 2010]. Indirectly, higher CO$_2$ levels are projected to lead to increased temperatures by 1.1 to 2.6 °C by the end of the century under the RCP4.5 scenario [Pachauri et al., 2014], resulting in the warming of the surface ocean and increased stratification of the ocean [Doney et al., 2009; Orr et al., 2005;

Pörtner et al., 2014]. These changes are expected to influence the marine biological pump, the inorganic carbon cycle and other biogeochemical cycles, but the extent of these changes is still little known.

Changes in the carbonate chemistry of the ocean have an impact on marine organisms in the upper levels of the ocean [Boyce et al., 2010]. Zooplankton play a fundamental role in the inorganic carbon cycle [Boyce et al., 2010; Henehan et al., 2016], specifically calcifying organisms such as foraminifera: microscopic unicellular protozoa. Planktic species are a significant globally important sink for $CaCO_3$, producing 32 to 80 % of $CaCO_3$ flux to sea floor sediments [Schiebel, 2002]. As planktic species have wide environmental ranges, as well as being abundant, they are a prime carrier of palaeoenvironmental proxies. Their calcite records past climates in its chemical composition, and assemblages move in response to environmental changes; such as variations in ocean circulation and depth [Armstrong and Brasier, 2005], tracking past climates. Therefore, any past foraminiferal calcification response to environmental change could provide an insight into how species may react in the future to a rapid change in atmospheric CO_2 and the implications to the biogeochemical cycle.

1.2. Environmental influences on calcification

Growth of foraminifera is fundamentally regulated by various environmental factors including pH, temperature, salinity, light, oxygen and nutrient levels, together with food availability [Kucera, 2007]. Larger test sizes are found in the warm subtropical to tropical oceans with decreasing size towards the poles [de

Villiers, 2004; Kucera, 2007; Schmidt et al., 2004b], differing with species. Increased test size in warmer surface waters is due to a combination of carbonate ion concentrations (CO_3^{2-}) saturation, faster metabolic rates, higher light intensity and greater niche diversity due to stronger stratification [de Villiers, 2004; Lombard et al., 2009; Schmidt et al., 2004b]; corroborating the suggestion of temperature being the dominant control on growth in lower latitudes [Bijma et al., 1990]. Strongly reduced salinity inhibits calcification in some species of foraminifera [Weinkauf et al., 2013]. With greater food and nutrient availability, size increases as less energy is needed to forage; though, shell size decreases above the optimum rate of primary productivity $150g/C/m^2/yr$ [Schmidt et al., 2004b]. These size trends across the group are the result of species-specific optimum growth conditions, outside of which growth rates decrease and reproduction ceases [Schmidt et al., 2008; Schmidt et al., 2004b].

Benthic foraminifera raise their internal pH levels during calcification, due to an up-regulating system which allows calcification in undersaturated waters [de Nooijer et al., 2009; Fabry et al., 2008], and therefore require more energy for growth in a high pCO$_2$ environment [de Nooijer et al., 2008; Foster et al., 2013]. It is unknown whether planktic foraminiferal species have a similar response. Nevertheless, planktic foraminifera still react to changes in carbonate chemistry with thinner tests and reduction in size [Bijma et al., 1999; Fabry et al., 2008]. This response is more prominent in non-symbiont foraminifera than symbiont species, which are also able to elevate the pH in surrounding waters up to one unit above seawater due to photosynthetic CO_2 fixation [de Nooijer et al., 2009;

Foster et al., 2013; Rink et al., 1998; Wolf-Gladrow et al., 1999].

While in the laboratory a clear reaction to carbonate ion changes is suggested, both modern and geological field studies of differing species of planktic foraminifera are more complicated. Over a range of ages and localities, size-normalised weight (SNW) shows reduced shell mass by up to 50% in response to increased CO_2, and therefore decreased CO_3^{2-}. This has been found in both recent field studies [Henehan et al., 2016; Moy et al., 2009; Osborne et al., 2016] and over a broad geological timescale [Barker and Elderfield, 2002; Gonzalez-Mora et al., 2008; Naik et al., 2010], as well as laboratory culture experiments [Henehan et al., 2016; Lombard et al., 2010].

Yet, Weinkauf et al. [2013; 2016] found other environmental factors control calcification, including temperature, productivity and optimum growth conditions, regardless of changes in CO_3^{2-}. Optimum growth conditions, in particular, can be coupled with seasonality; with a reduction in shell weight seen in *Globigerinoides ruber* as a result of increased upwelling [de Moel et al., 2009; Weinkauf et al., 2016]. Weinkauf [2016] found SNW of *G. ruber* to be lower in winter in comparison to the highest values in June and July. In contrast, SNW for *Globigerina bulloides* remained constant.

Results from these studies suggests the relationship between atmospheric CO_2 levels and weight of foraminiferal tests is species-specific and varies with location and environment, with some species showing no sensitivity at all [Davis et al., 2013; Henehan et al., 2016]. The same can be said for other biotic factors

that may control calcification, as all species have differing optimum growth conditions.

Overall, there appears to be no consensus on foraminiferal calcification response to changes in climate and many species responses are unknown. Further study, especially in a high CO_2 world, could help to provide insight into mechanisms driving foraminiferal size and weight to assist in building a coherent model of how this group could be affected by future warming and acidification.

1.3. The Pliocene environment

The Pliocene (5300 to 2800 ka BP, kiloannum or thousand years before present) was the last time CO_2 was as high as it is today, peaking at ~420 ppm [Dowsett and Cabellero-Gill, 2010; Pagani et al., 2010; Seki et al., 2010]. It is an interval comparable to the modern ocean, concerning basic ocean circulation and faunal distribution, [Robinson et al., 2008] and has climates similar to those projected for the end of the 21st century [Pachauri et al., 2014]. SST is estimated to be 2 to 3 °C higher, surface pH at ~0.06 to 0.11 units lower and sea level is ~25 m above current values [Badger et al., 2013; Haywood et al., 2009; Hönisch et al., 2012; Martínez-Botí et al., 2015; Pagani et al., 2010; Robinson et al., 2008; Seki et al., 2010]. However, this warm period was interrupted by a period of short-lived, global glaciation during the marine isotope stage (MIS) M2 (3260 to 3310 ka BP) [Dowsett et al., 2015; De Schepper et al., 2013]. M2 is the first large glaciation [De Schepper et al., 2013] and is thought to have been caused by increased circulation between the Pacific and Atlantic

oceans in response to the closure of the Central American Seaway (CAS) [Karas et al., 2016; De Schepper et al., 2013].

Thus, this time interval is ideal to quantify the response of planktic foraminiferal calcification to increasing temperature and ocean acidification, as a result of increased pCO$_2$. The hypothesis '*size-normalised weight of foraminifers will positively correlate with atmospheric CO$_2$ over the high amplitude of change seen in the Pliocene during MIS M2*' will be assessed in this project. Specifically, I will test the change in test size and SNW throughout the Pliocene of the planktic foraminifer *Globigerinoides ruber* (white) in the Caribbean. Micro Computer Tomography will be used to assess the causes of any weight changes. I will compare the results to changes in environmental parameters and previous studies of the Pliocene, conducted by Davis et al. [2013], and of weight and size data from higher latitude locations [Schmidt, unpub. data] to put my data into a more regional context. I will also be looking at the relationship between amplitude of change in the high CO$_2$ world to test if it is the same as in the cold world of the Pleistocene.

2. Materials and Methods

2.1. Materials

To measure the response of planktic foraminifera to increasing temperature and ocean acidification as a result of increased pCO$_2$, samples from ODP Site 999 were analysed (12°44.639'N, 78°44.360'W, 2828 m water depth) [Sigurdsson et al., 1996] (figure 1), located in the Caribbean Sea. Sedimentation and mass accumulation rates during the Pliocene at the site are recorded at 30 to 33 m/ma and 2.6 to 3.4 g/cm^2/ka, respectively [Sigurdsson et al., 1996]. The core is predominantly composed of nannofossil clayey mixed sediments with foraminifers and foraminiferal clayey mixed sediment with nannofossils [Sigurdsson et al., 1996]. 120 samples from core 11 section 2 to core 12 section 4 were used to focus on the late Pliocene and MIS M2. Sediment samples were washed over a 63 µm sieve and dry-sieved at 150 µm.
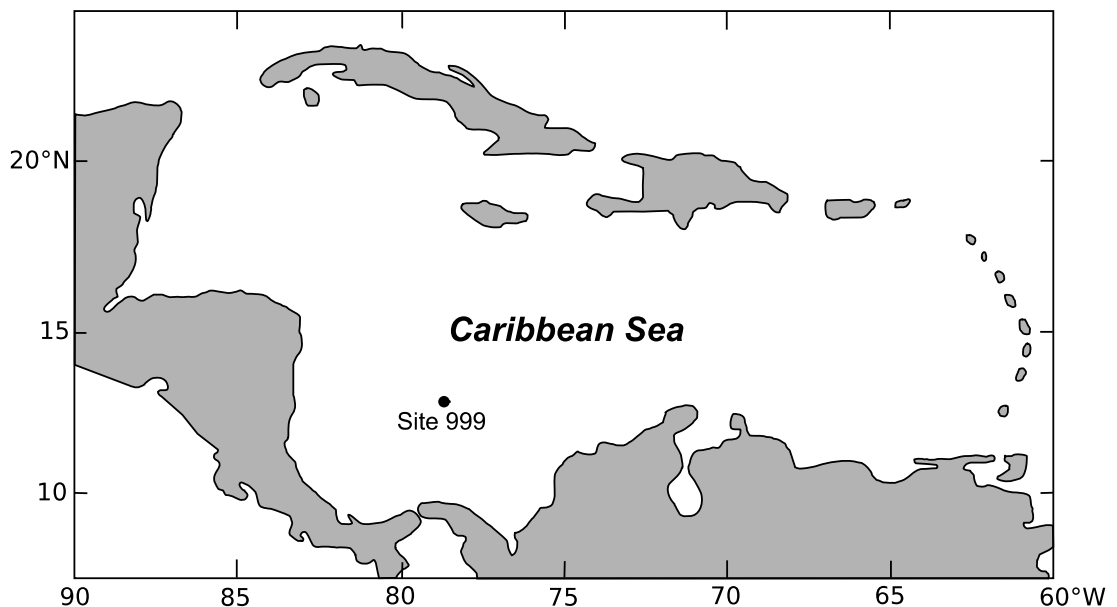
Figure 1. Location map of ODP Leg 165 Site 999 (adapted from Sigurdsson et al. [1996]).

The other sites used for comparison in this study are ODP Site 610A and IODP Site U1313. Site 610A is located in the Atlantic Ocean (53° 13.297'N; 18°53.213'W) and has a water depth of 2417 m [Ruddiman et al., 1983]. U1313

is situated at the base of the upper western flank of the Mid-Atlantic Ridge in a water depth of 3426 m [Channell et al., 2006].

The age model used has been calculated from Groeneveld [2005], which was initially established by Haug and Tiedemann [1998] (see Groeneveld [2005]). The sand percentage was taken from Haug and Tiedemann [1998]. Sea surface temperature data was calculated from Groeneveld [2005], based on planktonic foraminifera *Globigerinoides sacculifer* Mg/C ratios. Atmospheric CO_2 levels (pCO_2^{atm}) data is derived from boron isotope data in Martinez-Bolti [2015].

2.2. Methods

2.2.1. Automated microscopy

Planktic foraminiferal assemblage size of all 120 samples over 419 ka, resulting in a resolution of 3.5 ka on average, was determined through the analysis of community size generated by automated microscopy [Schmidt et al., 2004]. The samples were split representatively using a microsplitter so that ~1,000 to ~3,000 specimens of each sample were photographed. To calculate the size distribution within the assemblage, samples were analysed using AnalySIS (SISImage). Parameters were set to exclude particles with a maximum diameter below 200 µm (as whole tests were sieved over 150 µm which would exclude specimens with a minimum diameter of 150 µm), above sphericity of 0.5, and within the grey value of 47 to 220. Manual assessment of the images with the largest particle size removed any benthic foraminifera, ostracods, diatoms, and other material to avoid bias in the data. The 95th percentile was

calculated on the remaining size data. The average measurement error of the 95th percentile of the assemblage size (size[95/5]) is 2.14 %. This was determined by re-splitting and repeat measurement of some samples and calculated through equation 1 and 2. It gives an average error of 8.84 µm.

$$\text{Error (\%)} = \frac{size95/5_{first} - size95/5_{second}}{size95/5_{first}} \qquad (1)$$

$$\text{Average error (\%)} = \frac{Error\ (\%)}{n} \qquad (2)$$

2.2.2. Fragmentation

Dissolution of foraminiferal tests in corrosive bottom waters varies between species [Kucera, 2007] and can overprint the body size and weight that records the influence of the carbonate system of calcification in life post-mortem [Henehan et al., 2016; Kucera, 2007]. Fragmentation was counted in at least 200 tests and test fragments (defined as any test with damage to at least two chambers or having at least one chamber missing) [Davis et al., 2013]. Currently, the calcite lysocline is found at ~5 to 6 km in the Atlantic [Butler, 1991] and the drill site is at a water depth of 2828 m [Sigurdsson et al., 1996]. During the Pliocene, the lysocline was generally deeper than 4 km [Sigurdsson et al., 1996; Tiedemann and Franz, 1997].

2.2.3. Size-normalised weight

SNW is a measure of test wall thickness and density. To determine SNW of foraminifera through the Pliocene, 25 to 30 individuals of a species were picked from the 250 to 300 µm size fraction of 68 samples [Davis et al., 2013]. A narrow size fraction is used to help restrict mass variations due to test dimensions

[Barker and Elderfield, 2002]. *G. ruber* specimens were picked to reflect a specific morphotype, sensu stricto (s.s.). Other morphotypes are known to have different habitats and therefore potentially different reactions to environmental drivers [Thirumalai et al., 2014]; *G. ruber* sensu lato (s.l.) precipitate their shells in slightly colder surface waters than *G. ruber* s.s., and have been avoided accordingly [Steinke et al., 2005].

The samples were weighed using a six-decimal place balance (Mettler Toledo) to determine the average weight of the test, the most simple form of SNW otherwise known as the sieve-based weight (SBW) [Weinkauf et al., 2016]. In order to correct for size variability within the sample measurement-based weight (MBW), a more advanced method of SNW, was applied [Barker and Elderfield, 2002]. For every sample, the length of each specimen in the same orientation was taken from digital images using ImageJ. SNW for all samples was calculated by normalising SBW to the mean diameter for the corresponding size (eq. 2) [Barker and Elderfield, 2002]. SNW of the 68 samples has been combined with 50 more data points for ODP 999 from Davis et al. [2013], which were calculated using the same method.

$$SNW_{diameter} = \frac{mean\ SBW_{sample} \times mean\ diameter_{size\ fraction}}{mean\ diameter_{sample}} \qquad (2)$$

2.2.4. Tomography

15 specimens each were picked from 4 samples in the extremes of the oxygen isotope ($\delta^{18}O$) record before, inside and after MIS M2. Tomographic images were taken using a Nikon XT H 225 ST CT scanner (120kV, 58Va), configured

at 2.1 to 2.3 μm resolution, with an exposure time of 0.5 seconds and a total of 3141 projections. These images were processed in Avizo (Thermo Scientific™). The calcite to volume ratio was calculated (figure 2a and 2b) and the thickness of the penultimate chamber measured (figure 2c).
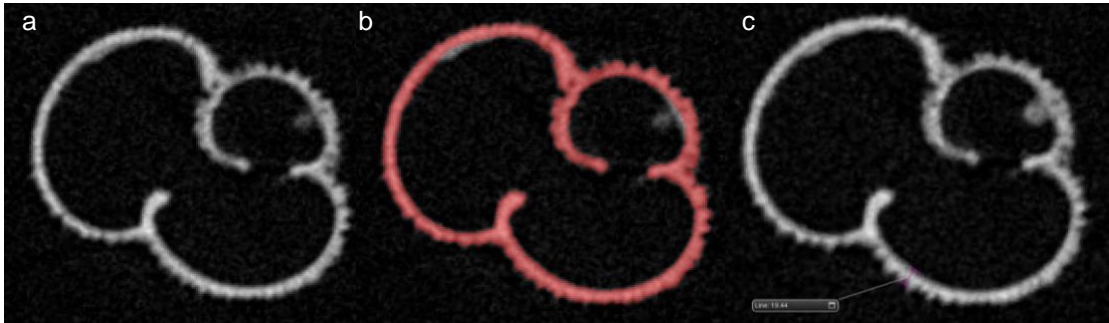
Figure 2. CT tomographic process: **a)** Raw image of one layer from micro CT scans, **b)** calcite test highlighted, after the removal of any other material, and **c)** test thickness of the penultimate chamber being taken using the line measuring tool.

3. Results
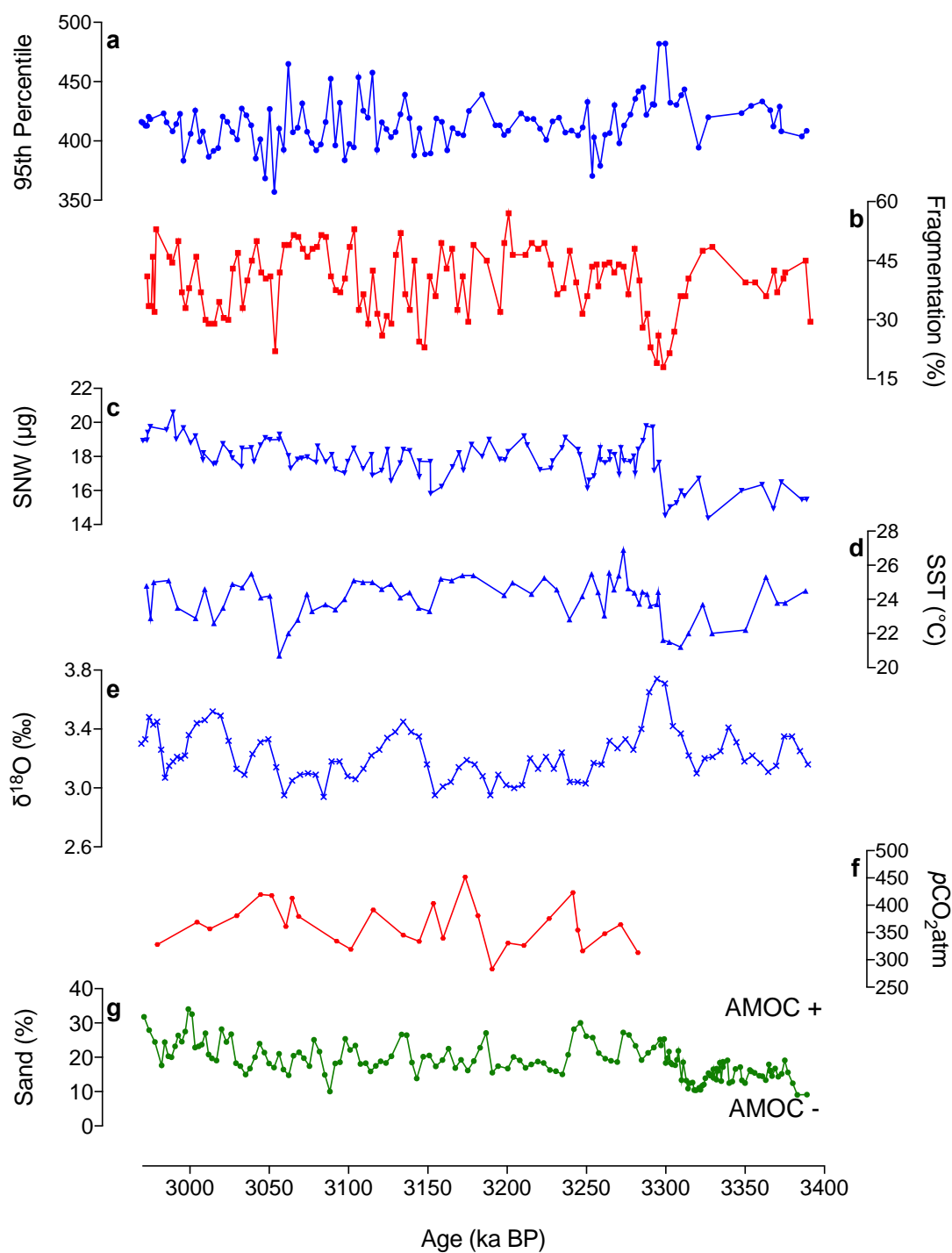
Figure 3. a) 95th percentile of the maximum diameter (μm) of planktic foraminifera, **b)** planktic foraminiferal fragmentation (%) (whole to fragment ratio presented as percentage), high values indicate more dissolution, **c)** size-normalised weight for planktic foraminifera *G. ruber,* combined with *G. ruber* SNW from Davis et al. [2013], **d)** SST (°C) from alkenone unsaturation index [Badger et al., 2013; J Groeneveld, 2005], **e)** LR04 benthic foraminifera oxygen isotope (δ18O (‰)) stack [Lisiecki and Raymo, 2005], **f)**

pCO$_2^{atm}$ determined from boron isotope data from ODP Site 999 [Martínez-Botí et al., 2015], and **g**) long term carbonate-sand record at ODP Site 999 [Haug and Tiedemann, 1998]. Grey bar indicates MIS M2 (3260 - 3310 ka BP) [De Schepper et al., 2013].
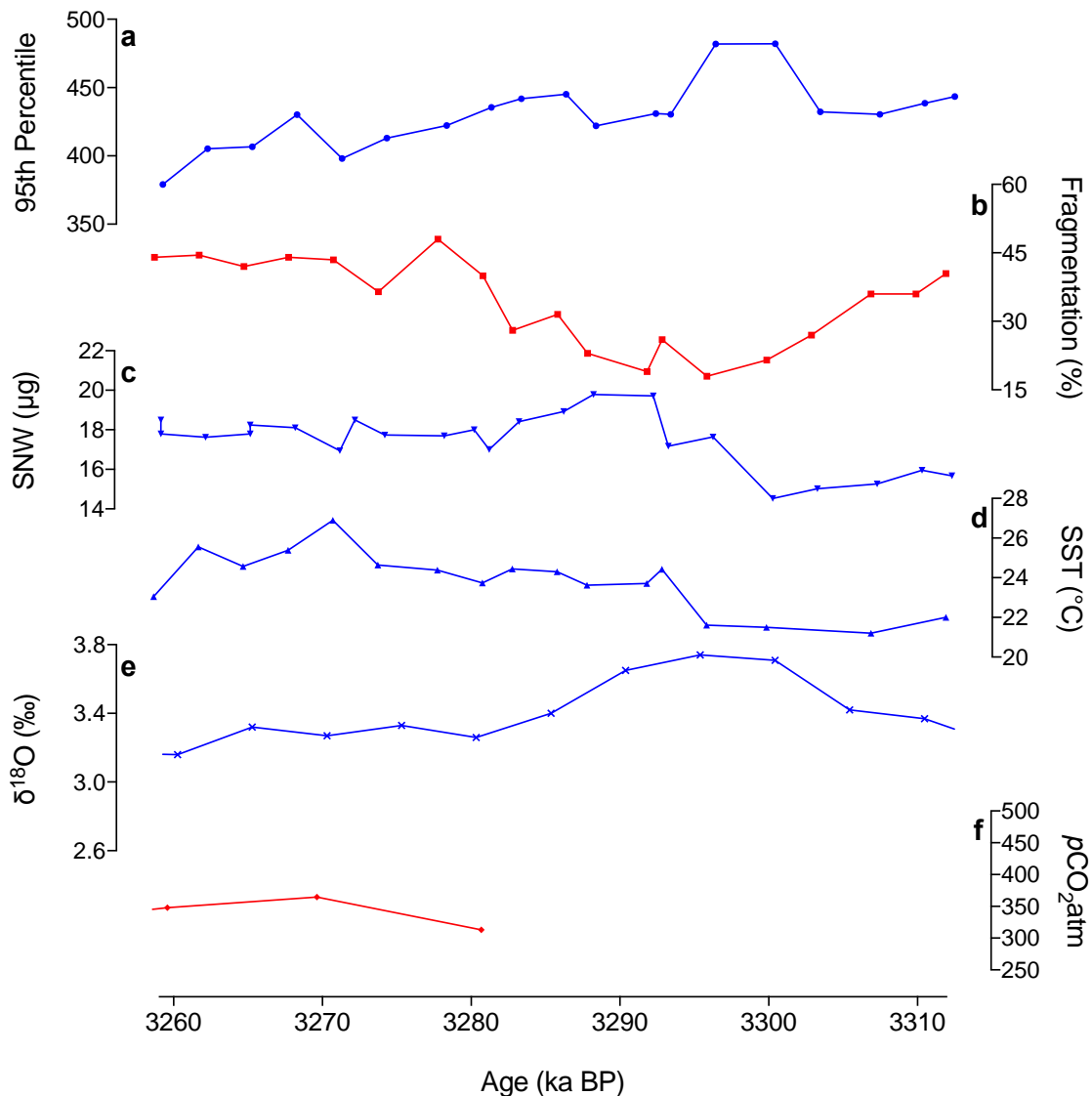
Figure 4. The above panels are for the duration of MIS M2, as indicated in figure 2 **a)** 95th percentile of the maximum diameter (μm) of planktic foraminifera, **b)** planktic foraminiferal fragmentation (%) (whole to fragment ratio presented as percentage), high values indicate more dissolution, **c)** size-normalised weight for planktic foraminifera *G. ruber,* combined with *G. ruber* SNW from Davis et al. [2013], also from ODP Site 999, **d)** SST (°C) from alkenone unsaturation index [Badger et al., 2013; Groeneveld, 2005], **e)** LR04 benthic foraminifera oxygen isotope (δ18O(‰) stack [Lisiecki and M E Raymo, 2005] and **f)** pCO$_2^{atm}$ determined from boron isotope data from ODP Site 999 [Martínez-Botí et al., 2015]. Long term carbonate-sand record [Haug and Tiedemann, 1998] does not change.

(caption continued on next page)

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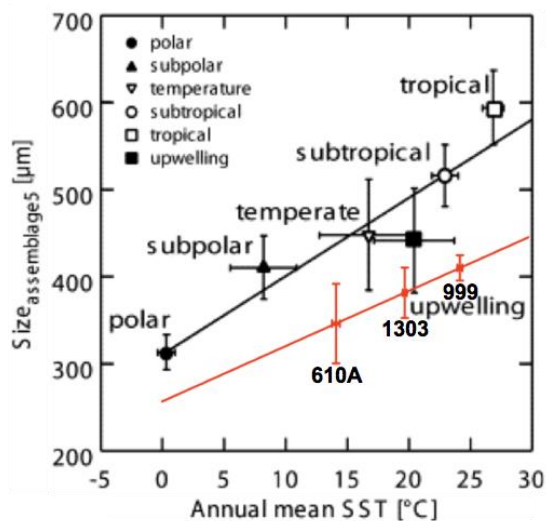
Figure 5. Holocene mean of size$_{assemblage5}$ (or size$^{95/5}$) (µm) per biogeographic area plotted against annual mean SST (°C). Error bars represent the 95%-confidence intervals. The black solid line corresponds to the regression line (r = 0.938, P = 0.006) for the Holocene. The red solid line corresponds to the regression line (r = 1, P = 0.0016) for the Pliocene. Data for ODP Site 610A and IODP Site U1313 taken from Schmidt [unpub. data]. Figure adapted from Schmidt et al. [2004b].
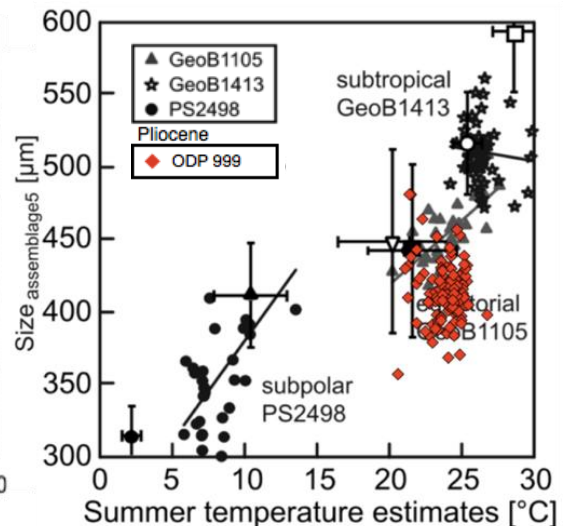
Figure 6. Late Quaternary size$_{assemblage5}$ (or size$^{95/5}$) (µm) compared to paleotemperature (°C) of all samples. Lines represent regression lines. Additionally, the mean size$_{assemblage5}$ per biogeographic zone of the Holocene are indicated. Error bars indicate the standard deviations for temperature and size$_{assemblage5}$. Figure adapted from Schmidt et al. [2003].

3.1. Fragmentation and dissolution

Fragmentation changes significantly at ODP 999 with a range of 30 to 60 % between 2970 and 3280 ka BP, and 30 to 50% prior to MIS M2 (figure 3b) suggesting a proximity of the lysocline. Fragmentation drops below 30% after 3280 ka BP showing increased preservation in MIS M2 (figure 4b). Fragmentation and SST are positively correlated (r = 016, P = 0.52), with higher fragmentation at warmer temperatures; higher temperatures suggest higher

$p\text{CO}_2^{atm}$ and therefore a shallower lysocline. Fragmentation and size are negatively correlated (r = -0.21, P < 0.0001), meaning change in size is not a result of dissolution.

3.2. Planktic foraminiferal size

$\text{Size}^{95/5}$ of planktic foraminiferal size ranges from 357 to 487 µm (figure 3a) and has a weak, negative correlation with SST (r = -0.035, P < 0.001) (figure 3f). Size and SST track each other after MIS M2 but not during the interval (figure 4a and 4f) with larger sizes recorded at cooler temperatures. $p\text{CO}_2^{atm}$ (figure 3e) and foraminiferal size are not correlated (r = 0.12, P = 0.13). To put the data into a regional context my data was compared to data from two higher latitude sites, ODP Site 610A and IODP Site U1313. The relationship between average size and temperature at each of these locations is positivity correlated (610A: r = 0.14, P > 0.001; U1313: r = 0.48, P > 0.001).

To assess if the size-temperature relationship is the same in a warmer world compared to today the Pliocene data was compared to Holocene biogeographic trends [Schmidt et al., 2004b]. While the trends are similar, the gradient of the line is flatter, and the size is smaller at the same temperature (figure 5). A comparable relationship between size and paleotemperature can be seen when size data from ODP Site 999 was plotted with Late Quaternary data covering the last 300 ka [Schmidt et al., 2003] (figure 6). ODP 999 plots within the equatorial range of modern planktic foraminifera, albeit Pliocene specimens, appear to have a decreased size in comparison suggesting an evolutionary offset.
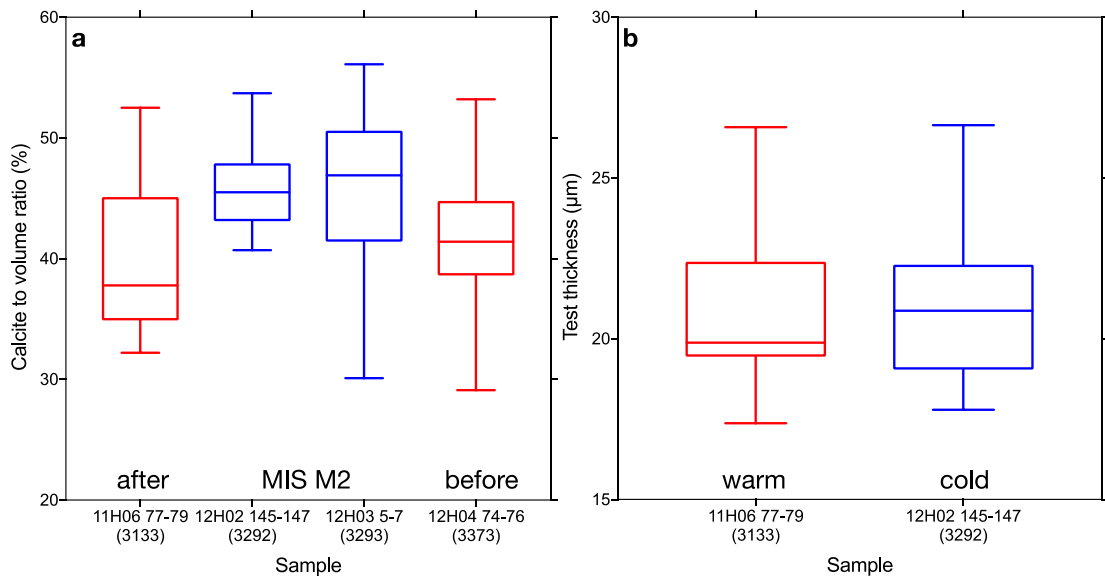
Figure 5. Drivers of change in calcification: **a)** calcite to volume (CV) ratio (%) of *G. ruber* from both warm (red) and cold (blue) samples of the Pliocene, and **b)** test thickness of the penultimate chamber from samples with the most extreme mean CV ratio measurements.

3.3. Size-normalised weight

Weights range from 14.4 to 20.0 µg for *G. ruber* (figure 3c) with a relatively low variability. At 3300 ka BP test weight drops, which cannot be due to dissolution as preservation is at its best. The decrease in SNW is observed at the lowest temperatures during MIS M2 (figure 4c), with SNW and temperature being positively correlated (r = 0.31, P > 0.001). Yet, as temperature increases during the glacial period of MIS M2, there is a peak in SNW. Both temperature and size SNW remain stable after this event.

Calcite to volume (CV) ratios of *G. ruber* during the Pliocene (figure 7) shows increased calcification during the cooler MIS M2. The average percentage of calcite to volume was 45.9 % and 46.1 % for 3292 and 3293 ka BP, respectively compared to 40.3 % before MIS M2 and 41.0 % after. There is a delay of 7 ka between the measured peak weight and peak $\delta^{18}O$ (‰). For weight, there is a sample resolution of 6.1 ka and for $\delta^{18}O$ resolution is at 4.6 ka.

Test thickness was quantified in the highest and lowest CV ratio samples showing a marginal difference of 0.41 µm between the warm and the cold samples. There is no significant difference between the results for 12H02 145-147 (M = 21.10, SD = 2.55) and 11H06 77-79 (M = 20.69, SD = 2.34) test thickness (t (28) = -0.003, P = 0.99). The accuracy of results (s = 0.31) was calculated from 10 repeat measurements of the same specimen in one sample.

4. Discussion

4.1. Factors influencing foraminiferal assemblage size

Foraminifera at ODP Site 999 are larger and more fragmented during the cooler temperatures of MIS M2, after which they became larger and more fragmented when warmer. During MIS M2, the size declines overall, despite a general warming trend. Fragmentation does not couple with foraminiferal size at ODP Site 999 and is therefore not causing bias in results. Size is reflecting a response to ecological changes, rather than dissolution. Results can, therefore, be deemed reliable.

Size changes in planktic foraminifera are related to ecological conditions such as salinity, light, oxygen and nutrient levels, temperature and carbonate saturation [Kucera, 2007]. Against the expectations of seeing larger test size in warmer temperatures, an increase in assemblage size is observed during the coldest phase of MIS M2 where pCO$_2$ would be at its lowest. Assemblage size shows high frequency variability throughout the Pliocene which is not related to either carbonate chemistry or temperature; these are generally stable throughout.

With no correlation between pCO$_2{}^{atm}$ and foraminiferal size and with SST also not tracking pCO$_2{}^{atm}$ locally both are potentially responding to the changes in water flow across the Central American Seaway (CAS) and consequentially alterations to American Meridional Overturning Circulation (AMOC), rather than atmospheric pCO$_2$. Overall shoaling of the thermocline was triggered by increased AMOC ~ 4800 to 4000 ka BP, initiated by the progressive closure of

the Central American Seaway (CAS) [Steph et al., 2010]. The intensification of glaciation initiated at ~3600 ka resulted in increased upwelling [Steph et al., 2010] and provides an explanation for increased foraminiferal test size during MIS M2.

However, following MIS M2, carbonate dissolution increased at ODP Site 999 due to the Antarctic Intermediate Water (AIW) entering into the Caribbean Basin in place of North Atlantic Deep Water (NADW), during an open CAS [De Schepper et al., 2013]. This created weak overturning circulation [De Schepper et al., 2013; Haug and Tiedemann, 1998], and cooling is cut short, causing increased stratification. Changes to foraminiferal habitat through variations in stratification, and thus temperature, can affect assemblage size by altering the faunal composition [Schmidt et al., 2003].

Consequently, there is a decrease in size, despite the warmer temperatures and the relationship observed reflects an increase in stratification, which has previously been linked to the increased size of foraminiferal tests [Wei, 1994]. Fluctuations in AMOC would, therefore, explain the relationships between size and temperature during the Pliocene; which is the reverse of what is normally seen in planktic foraminifera [Schmidt et al., 2004b]. It also suggests that planktic foraminiferal size is responding to changes in ocean stratigraphy rather than pCO_2; although temperature is a controlling factor.

4.2. Impact of amplitude of change and implications of evolution

Over the time in this study, pCO_2 appears relatively stable with ~40 ppm

variability between 40 ka glacial-interglacial periods [Badger et al., 2013; Davis et al., 2013]. In contrast, during the Pleistocene pCO_2 varied by ~90 ppm variability over 50 ka periods [Martínez-Botí et al., 2015], during which planktic foraminiferal size variability (378 to 520 µm) is mainly affected by temperature [Schmidt et al., 2003]. During the Pliocene, temperature and size are negatively correlated but in the Pleistocene, there is a positive correlation (r = 0.16, P > 0.001). The change in size of foraminifera is, therefore, greater at higher amplitudes of temperature change. With both the Pliocene and Pleistocene being considered, the degrees of warming in comparison to the difference in size scales with a linear correlation [Schmidt et al., 2003]. This relationship indicates a match between change in foraminiferal size and amplitude of palaeoclimatic fluctuations, with no significant variation in stable environments but substantial change in those that are highly variable [Schmidt et al., 2003].

In general, Pliocene foraminifers are smaller than in the Pleistocene. Tropical Pliocene foraminifera are also smaller, by ~50 to 100 µm, in comparison to the modern. This unprecedented increase in size has been found to be a trend by Schmidt et al. [2004a], with assemblage size in tropical locations increasing overall from ~490 to 550 in the early Pliocene to ~540 to 600 µm in the modern [Schmidt et al., 2004a]. This is found despite a drop in size throughout the Pliocene before the rapid increase that commences at the Pliocene/Pleistocene boundary. Nevertheless, it is concluded environmental change was the cause of increased size in whole assemblages, as well as at a species level [Schmidt et al., 2004a].

A comparison of the Pliocene planktic foraminiferal size and temperature relationship to the Late Quaternary (figure 5 and 6) shows that not only are the assemblages smaller but the relationship between size and temperature has a flatter gradient than that of the modern. This may be a result of foraminifera reducing in size as a response to the warmer ocean during the Pliocene. This evolutionary signal implies a difference between a warming world and a cool one, where larger increases in pCO$_2$ over a smaller timescale causes an intensified reduction in calcification. This further indicates rapid warming has a greater influence on foraminiferal size than what is found in the Pliocene.

4.3. Calcification and size-normalised weight changes

Increasing fossil fuel burning is projected to lead to a decline in shell weight and calcification in planktic foraminifera of 4 to 8 % and 6 to 14 % at 560 and 750 ppm CO$_2$ respectively [Doney et al., 2009]; levels projected to be reached by 2050 and 2100. Culture experiments [Bijma et al., 1999; Spero et al., 1997] of *Orbulina universa* and *Globigerinoides sacculifer* show a decrease in shell weight and calcification with lower carbonate ion concentrations. Barker and Elderfield [2002] reported similar results, from North Atlantic samples across glacial-interglacial Termination I, for *Globorotalia truncatulinoides*, *Globorotalia inflata*, *G. bulloides*, and *Neogloboquadrina pachyderma* to CO$_3^{2-}$ and SST. The response was species specific, however. Similarly, Naik et al. [2010] found this relationship in *G. sacculifer* and *G. ruber* in the Arabian Sea during the Holocene and the Last Glacial, and Osborne et al. [2016] found CO$_3^{2-}$ saturation state to be the primary control for calcification in modern foraminifera of the Santa Barbara Basin. From the Madeira Basin, Weinkauf et al. [2016] too found

a relationship between CO_3^{2-} and growth, similar to those in the studies mentioned above, in modern samples of *G. ruber* and *Globeriginoides elongatus.* There was no environmental forcing in *G. bulloides.* It is worth noting the different methods used for SNW between current available findings is not always comparable. This should be considered in future studies, especially as SNW of planktic foraminifera is becoming an increasingly important palaeo-proxy for marine carbon chemistry [Beer et al., 2010].

A recent study of Pliocene planktic foraminifera from ODP Site 999 during the drop in pCO$_2$ by Davis et al. [2013] revealed that *G. bulloides* and *Globorotalia punctulata* have varying test weights with a possible correlation to temperature in the North Atlantic*. G. ruber*, on the other hand, shows no change in weight throughout the same period. Yet, this study has found *G. ruber* to have a significant relationship with temperature, but not pCO$_2$; though, this may be a result of insufficient pCO$_2$ data for the Pliocene. SNW of *G. ruber* decreases during MIS M2 and is, therefore, heavier at higher temperatures. There is a large, increasing step in SNW during MIS M2, parallel to warming, thus challenging the expected results of decreased SNW during increased temperatures and thereby for inference higher CO$_2$. This suggests calcification is being controlled by another factor, such as productivity or temperature. Both of these environmental controls are found to be correlated with SNW by Weinkauf et al. [2016] in the modern in both *G. ruber* and *G. bulloides* and temperature in the same species in the Late Quaternary and Gonzalez-Mora et al. [2008].

Furthermore, there is a delay between peak weight and peak oxygen levels during MIS M2. Although this data is high resolution, the difference in interval times between the two records could result in bias, showing an offset between the peaks. If the offset is not due to bias, it may be showing weight change in *G. ruber* was not instantaneous with warming events. This indicates the species were able to adapt to increased sea surface temperatures as a result of the relatively low rate of change. To ascertain if this is the case, bias within the records would need to be removed by further increasing the resolution of all data sets.

Weight change in this species is not driven by test thickness (figure 7), which remains relatively stable, but rather variability in CV ratio. This suggests CV ratio changes are being generated by increased calcification elsewhere in the foraminiferal test. End-stage thickening in *Neogloboquadrina dutertrei* means older chambers are more heavily thickened than later chambers [Fehrenbacher et al., 2017] and although has not yet been confirmed that this occurs in *G. ruber*, it could provide an explanation for increased SNW as measurements were taken from the penultimate chamber. Changes could also be caused by either increased density [Beer et al., 2010] or an increase in the number of chambers formed during growth. All are factors which can be tested in the future to explain changes in CV ratios. Although, CV ratios would not be affected by this, there is also the chance precipitation of inorganic secondary calcification could be influencing the outcome of SNW [Weinkauf et al., 2013]. This is unlikely as specimens appear relatively clean, with the occasional partial infill of chambers seen during tomographic analysis, and are well preserved.

Morphology could also have an impact on both size and weight results. *G. ruber* has significant morphological variability (see appendix A1); morphotypes are known to have differing ecological preferences [Bonfardeci et al., 2018; Caromel et al., 2015] and have the potential to lead to misinterpretations of data if not accounted for. As stated previously only s.s. morphotypes were proposed to be picked (see 2.3), to reduce error within the measurements taken. Though, there is still some variation within the picked s.s. specimens (see appendix A1 and A2) that could cause bias in the data. Some *G. ruber* kummerforms may have been picked inadvertently, negatively impacting results; these do not appear in the small sample size picked for comparison (appendix A2) and needs further exploration.

Largely, the results of this study contradict previous outcomes and the hypothesis on the relationship of both size and weight with environmental parameters, including pCO_2, in *G. ruber*. I suggest this is fundamentally down to amplitudes of change in pCO_2 during glacial-interglacial periods, as well as secondary controls such as temperature and optimum growth conditions. To help predict future foraminiferal responses to a changing climate, an investigation into long-term trends in pCO_2 and CO_3^{2-} would need to be conducted, in addition to clarifying species-specific controls on calcification.

5. Conclusions

Planktic foraminiferal size fluctuations responses to temperature changes during the mPWP and follow the global trend of increased size during warmer temperatures. This relationship changes at MIS M2 due to alterations to AMOC owing to the shallow openings and closures of the CAS. As a result, stratification and nutrient availability become the dominant factor in the size of foraminifera.

SNW in *G. ruber* suggests calcification is not affected by pCO$_2$. Temperature seems to be the dominant control and while there is a clear correlation with weight, the results were not as expected; with a reverse relationship found between size and temperature. Therefore, secondary controls are also likely to be important: environmental parameters such as nutrient availability or salinity may be affecting calcification.

The fact pCO$_2$ is not the controlling factor during the Pliocene suggests the rate of change over glacial-interglacial periods may have a pronounced impact on the response of planktic foraminiferal calcification; with a more rapid increase in pCO$_2^{atm}$, there is a greater decline in calcification. The comparison between a stable Pliocene and a more variable Pleistocene supports this. Moreover, considering a comprehensive literature review and the results of this study, it is clear that response to changes in climate is species-specific. It should also be pointed out that a more extensive, higher resolution study of SNW.

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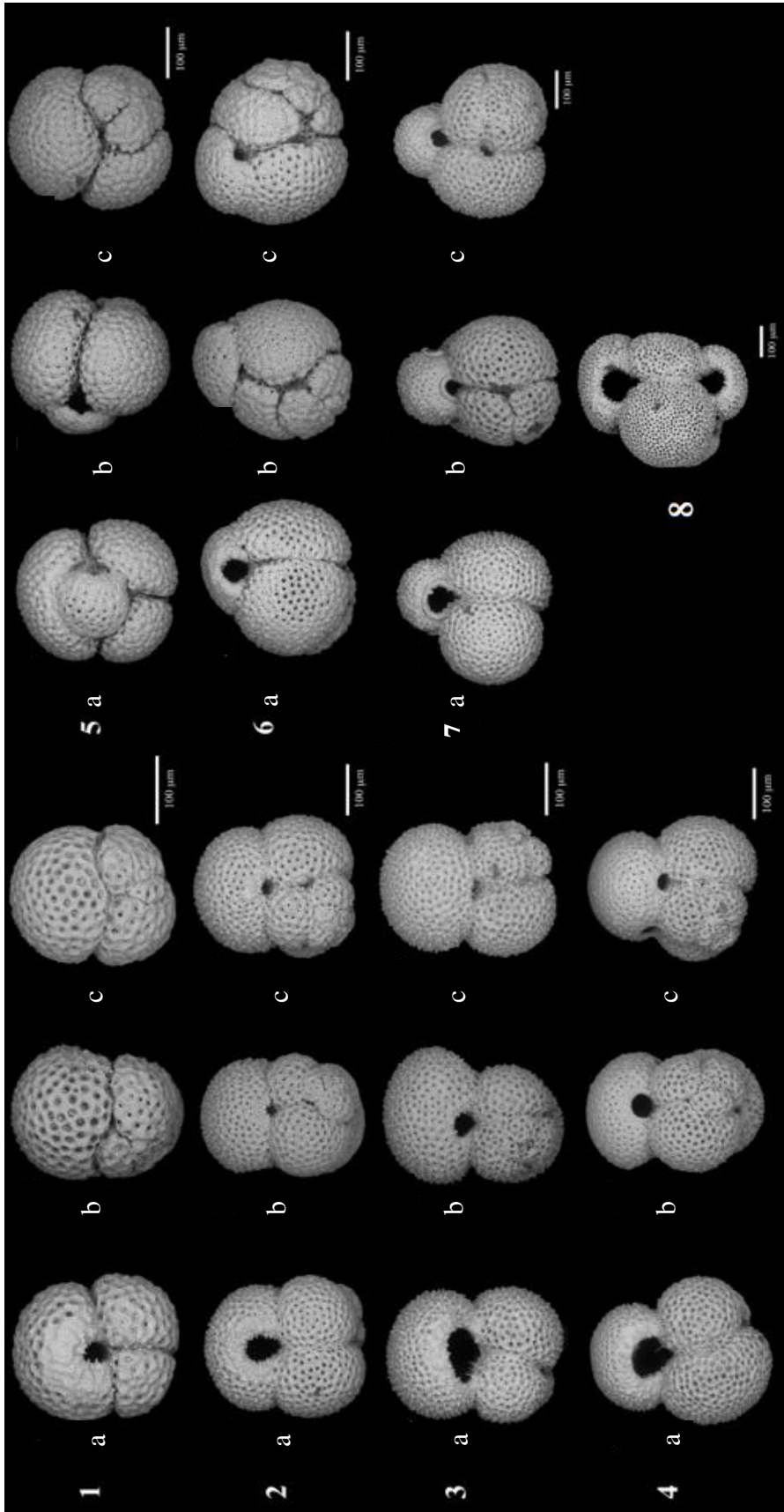
Appendix

Appendix A

Figure A 1. (a) umbilical side view, **b)** side view, and **c)** dorsal side view). **1-2)** *Globigerinoides ruber* cyclostome type, **3-4)** *G. ruber* sensu stricto, **5-7)** *G. ruber* kummerform group, **8)** *G. ruber* "helicina" type. Figure adapted from Bonfardeci [2018].
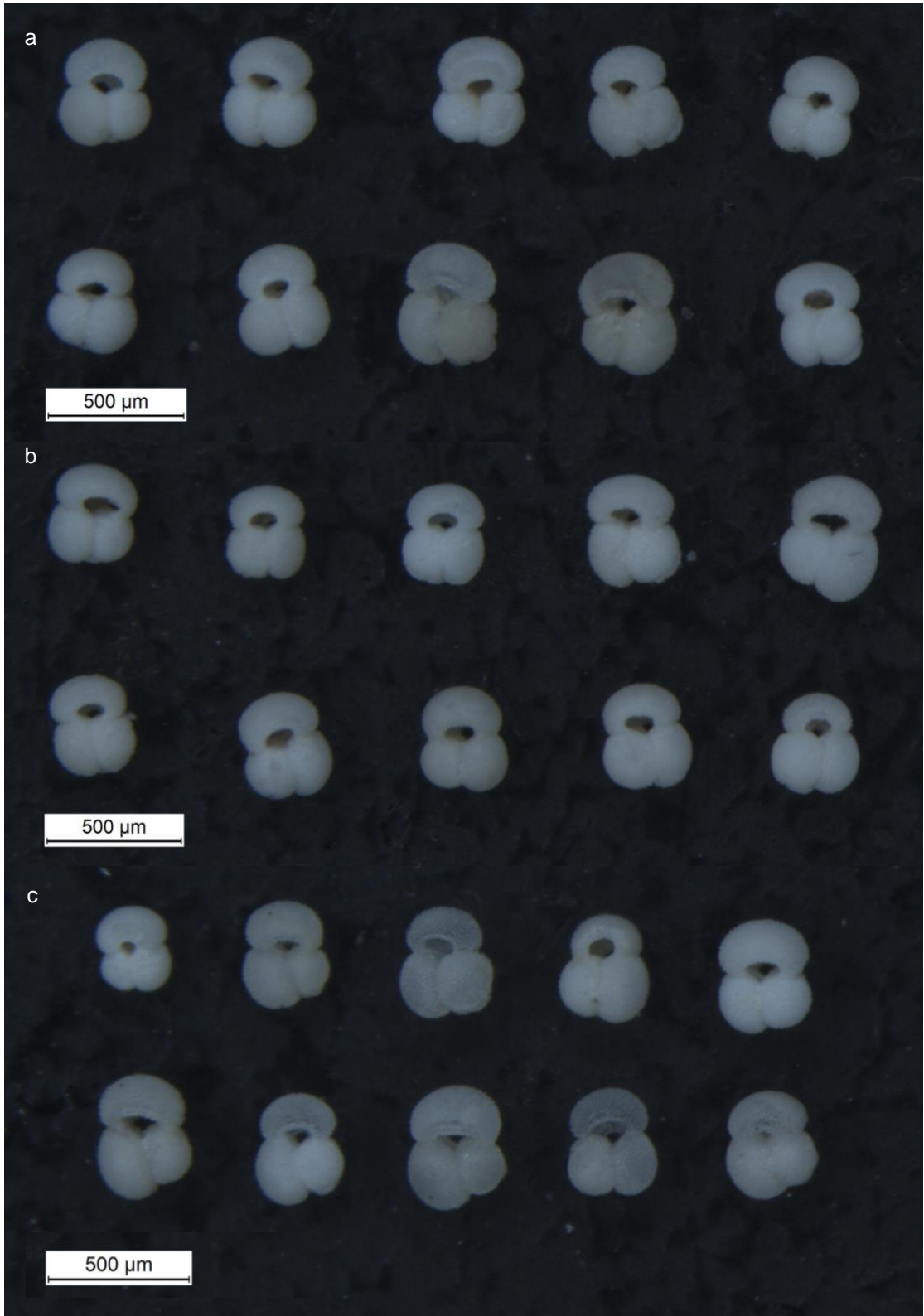
Figure A 2. (umbilical side view) *G. ruber* specimens from **a)** sample sample 11H06 77-79 (3133 ka BP, after MIS M2), **b)** sample 12H03 5-7 (3293 ka BP, within MIS M2), and **c)** 12H04 74-76 (3373 ka BP, before MIS M2) of ODP Site 999 cores.