The Controls on Biogeochemical Proxies and Shell Calcification in Modern Planktonic Foraminifera

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This Dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in 'contributions' at the end of each chapter.

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Abstract

Planktonic foraminifera (PF) shell biogeochemistry can be used to reconstruct past oceanic and climatic changes, but the influence of other environmental and biological interactions can compromise their use as reliable proxies. This study aims to develop their use as biogeochemical proxies by determining the primary controls on shell flux, morphometric parameters and geochemical variability in modern PF, and apply this to understand how recent anthropogenic ocean acidification has affected their calcification since the ‘pre-industrial’.

By using PF from a biweekly sediment-trap time series (years 1998-2000 & 2008-2010), with nearby monthly hydrographic data from the Sargasso Sea in the oligotrophic North Atlantic, this study shows that PF contribute up to ~40% of the calcium carbonate flux in the Sargasso Sea during the winter months when the mixed layer is deepest. Therefore factors affecting the mixed layer dynamics such as the North Atlantic Oscillation could potentially regulate PF and carbonate flux on decadal timescales. Shell calcification, estimated from shell morphometrics of two species (*Globigerinoides ruber* (pink) and *Orbulina universa*) is primarily controlled by temperature and [CO$_3^{2-}$], rather than dependent on optimum growth conditions. Biological and calcification processes appear to significantly affect the trace element concentrations in PF; this study shows for the first time that calcification rate controls boron incorporation whereas growth processes appear to control uranium incorporation in *G. ruber* (p), *O. universa* and *Globorotalia truncatulinoides* (non-encrusted), suggesting that B/Ca and U/Ca are not reliable palaeo-pH proxies. The separation of morphotypes could vastly improve the accuracy of all PF trace element calibrations. Finally, by comparing the calcification of PF in the modern sediment trap samples with ‘pre-industrial’ surface sediments collected nearby in 1875, this study reports a 17-23% reduction in shell calcification of *O. universa* and *Globorotalia inflata*, caused by anthropogenic ocean acidification in the subtropical gyre. This inhibition in calcification is equivalent to a ~24 μmol/kg reduction in [CO$_3^{2-}$], consistent with the modelled [CO$_3^{2-}$] reduction since the ‘pre-industrial’.
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1. Introduction

1.1. Biology

Planktonic foraminifera are microscopic, calcium carbonate-shelled animals (Protozoa) ranging between ~100-1000μm in size, which are hugely abundant in the upper ~800m of both tropical and polar oceans (100-1000 ind./m³) (Schiebel and Hemleben, 2005). There are around 50 extant species of planktonic foraminifera (Kucera, 2007), which biologically precipitate new calcite (CaCO₃) chambers episodically in a spiral structure, with each successive chamber generally becoming larger than the previous (Hemleben et al. 1989). The geometric arrangement of these spirally structured shells forms the primary basis for taxonomic identification. However, subsequent work has shown that for particular species, genetic sequencing is necessary to classify genotypes, re-defining originally classified species (Darling et al. 1999, Darling and Wade 2008, de Vargas et al. 1999, Morard et al. 2009, Aurahs et al. 2009, Morard et al. 2013). Outside of the shell, cytoplasm is stretched into long strands forming the rhizopodia network, which collects food particles for herbivorous, omnivorous and carnivorous species and in some species generates food via photosynthetically active symbionts. Living specimens are typically heterotrophs, living off organic matter and exist in the subsurface ocean, but some live in the euphotic (light saturated) zone due to the presence of their photosymbiotic algae (Hemleben et al. 1989) and typically survive between a few days to a year. There is convincing evidence of a lunar to semi-lunar reproductive cycle for some species (Spindler et al. 1979, Bijma et al. 1990, 1994, Jonkers et al. 2015). Although others such as the *Globorotalia truncatulinoides* appear to follow a seasonal cycle, reproducing in the surface waters before descending up to 800m whilst growing from juvenile to adults (McKenna and Prell, 2004). Each species of planktonic foraminifer favours specific ecological conditions which determines their habitat depth e.g. in the euphotic (light-saturated) zone vs. thermocline (subsurface ocean), and also their preferred oceanographic setting e.g. warm, oligotrophic (nutrient poor) waters vs. cooler, upwelling (nutrient rich) waters.
1.2 Global Significance

Planktonic foraminifera make up essential parts of the marine ecosystem and typically contribute up to 56% of the open ocean marine calcite flux and up to 80% of the deep marine calcite budget (Schiebel, 2002). As calcium carbonate also ballasts the majority of organic carbon flux from the surface to the deep oceans (Armstrong et al. 2001, Klaas and Archer, 2002), planktonic foraminifera are globally important to both organic carbon and carbonate cycling in the ocean. Given that the invasion of anthropogenic carbon dioxide (CO₂) into our oceans is causing an ongoing reduction in calcium carbonate saturation, ocean acidification poses a real threat to planktonic foraminifera and other calcifying organisms (Feely et al. 2004, Orr et al. 2005, Moy et al. 2009) so it is imperative to monitor how their calcification has changed over the past ~150 years.

1.3 A Palaeoceanographic Toolbox

Whilst planktonic foraminifera are obviously vital parts of the ecosystem and biogeochemical cycling, they also act as important tools in palaeoceanography. Each species of planktonic foraminifera tends to favour a specific set of ecological and environmental conditions, which is incorporated in the shell during its precipitation. This environmental imprint recorded during growth and calcification, makes various aspects (e.g. species abundance, shell morphometrics, and shell geochemistry) of planktonic foraminifera excellent ‘tools’ with which to understand past environmental change. A better understanding of past changes in seawater properties inferred from these foraminifera-based proxies, will equip us with knowledge to understand the impact of current and future climatic change.


Despite the wide application of these proxies, numerous studies have demonstrated complicated relationships involving other environmental and ecological controls on species abundances, calcification and hence trace element incorporation in planktonic foraminifera. For instance, light intensity (for symbiont-bearing species) has been shown to affect the species abundance (Ortiz et al. 1995, Kuroyanagi and Kawahata, 2004), shell size (Caron et al. 1981, 1987, Bijma et al. 1992, Ortiz et al. 1995), shell calcification (Bijma et al. 1999, Lombard et al. 2010), and potentially trace element incorporation (Babila et al. 2014) into planktonic foraminifera. Similarly, some studies have observed planktonic foraminifera reaching their maximum abundance and size when conditions are 'optimal' to growth (Hecht, 1976, Bijma et al. 1992, Schmidt et al. 2004) i.e. a combination of optimal temperature, carbonate chemistry, light intensity, salinity, primary productivity (Fairbanks and Weibe, 1980, Northcote and Neil, 2005, Storz et al. 2009), and water column stability (Thunell and Reynolds, 1984, Lohmann and Schweitzer, 1990, King and Howard, 2003). There is some evidence to suggest that planktonic foraminifera growing under optimum conditions may also have faster calcification rates supported by the association of thicker shells in concurrence with maximum abundance (de Villiers, 2004) and increased growth rates (Aldridge et al. 2012). On the contrary, another study finds large inter-species variability in shell thickness and hence calcification controls, with no correlation with species flux and hence optimum growth conditions (Beer et al. 2010). Methodological differences in measuring shell thickness could explain some of the inter-species discrepancies, with sieve-size methods not as effective at normalising shell
weight than measured shell sizes (Beer et al. 2010). Alternatively, other controls may govern the calcification of different species such as temperature (Gonzalez-Mora et al. 2008, Marr et al. 2011), nutrient availability (Aldridge et al. 2012) or genetic variability within species, manifested as morphologically distinct varieties (Darling and Wade 2008, Marshall and Thunell, 2014).

Foraminiferal geochemical proxies often undergo progressive stages of 'confidence' during development, graphically displayed in Figure 1.1:

![Figure 1.1. The Palaeoceanographic Proxy Confidence Factor Phase Chart. Initially a new proxy emerges but as it is explored in more detail, problems emerge and confidence in the proxy dwindles. As these problems are gradually addressed through more research, confidence begins to rise and plateau into realistic expectations. Taken from Elderfield (2002).](image)

Consequently, proxies that lie in the 'realism' phase of Figure 1.1, tend to be reliable recorders of past environmental conditions but also have defined limitations. For example, foraminiferal stable isotopes were exploited by Emiliani in 1954 and have proved useful recorders of glacial-interglacial cycles and orbital cycles (Emiliani 1954a, 1954b, Shackleton and Opdyke, 1973) even though they can be affected by $[\text{CO}_3^{2-}]$ (Spero et al. 1997). Likewise, the concentration of the trace element 'Mg' in foraminiferal
calcite (Mg/Ca) has been shown to predominantly reflect temperature changes (Elderfield and Gannsen, 2000, Anand et al. 2003), but can also be affected by extreme lows in $[\text{CO}_3^{2-}]$ (Russell et al. 2004), high salinities (Ferguson et al. 2008, Kisakürek et al. 2008) and post-depositional dissolution of the shell (Dekens et al. 2002, Rosenthal and Lohmann et al. 2002). Other geochemical proxies are still undergoing active development; for instance, oceanographers are particularly interested in $\delta^{11}\text{B}$ and B/Ca because they have shown promise as potential palaeo-pH indicators (Hönisch and Hemming, 2004, Allen et al. 2011, 2012, Henehan et al. 2013, Babila et al. 2014, Henehan et al. 2015) and hence could help us understand the magnitude and rate of current ocean acidification in context with past events.

Other trace elements such as U/Ca, Li/Ca, Sr/Ca, could also be useful for evaluating past carbonate chemistry, temperature, or foraminifera calcification rate but the effect of additional independent variables on the uptake of these trace elements into foraminiferal calcite is still uncertain (see Table 4.1, Chapter 4). This is further compounded by the unknown extent to which biological processes (such as biomineralisation, kinetic effects, metabolism, respiration, photosymbiosis and reproduction), and life history (such as diet, depth habitat and seasonality), termed 'vital effects' (Urey et al. 1951, Erez, 1978), influence geochemical signals. Until there is a mechanistic description of foraminiferal calcification, the biological processes behind the cause of this uncertainty are unlikely to be resolved, but their influences can, and should, still be quantified.

A recent review on the use of foraminiferal B/Ca as a seawater carbonate chemistry indicator, suggested that biological processes may influence B uptake and this should be investigated along with the effect of shell size and species-specific relationships on B incorporation (Allen and Hönisch, 2012). The need for a better understanding of the biological influence on foraminiferal trace element concentrations have been echoed in other geochemical studies which have also reported shell-size dependent trace element concentrations in certain species (Elderfield et al. 2002, Ni et al. 2007, Friedrich et al.
2012, Henehan et al. 2013, 2015, Babila et al. 2014, Ezard et al. 2015), currently attributed to changes in growth (Schmidt et al. 2008) and hence calcification rates (Ni et al. 2007). Finally, the importance of genotypic and morphotypic variability on trace element and stable isotope composition needs to be established as previous studies have found both significant (Wang, 2000, Steinke et al. 2005, Henehan et al. 2015) and insignificant effects of morphological variability (Thirumalai et al. 2014) on chemical composition of foraminiferal calcite.

It is clear from this brief evaluation that the interpretation of foraminiferal proxies are complicated by the balance and interaction of various ecological and environmental variables. Whilst culture studies can tease out the primary controls on certain proxies e.g. calcification (Bijma et al. 2002), trace element geochemistry (Russell et al. 2004, Allen et al. 2011, 2012), open-ocean studies are more geared to testing the effects of multi-stressor environments on planktonic foraminifera species abundances, shell parameters and geochemistry. Population growth (measured through species abundance), shell growth/calcification (measured through shell size/thickness) and geochemistry (trace element and stable isotope) are inherently linked in the lifecycles of planktonic foraminifera, but most studies only measure only one or two of these processes when attempting to understand the controls on their variability.

The aim of this study is to develop multiple proxies on the same open-ocean sample-set, in order to assess the full ecological response of different planktonic foraminifera species to a combination of environmental variables on seasonal and interannual timescales. I will then apply new understanding of biogeochemical proxies in planktonic foraminifera to assess the effect of anthropogenic ocean acidification on their shell calcification.
1.4. This Study

Firstly, I attempt to characterise the complex relationships between multiple planktonic foraminifera proxies mentioned above (i.e. shell flux, size, thickness, stable isotope and trace element geochemistry) and concurrent physical/chemical/biological changes in the water column. To do this, I will use bi-weekly sediment traps collected at 1500m, in conjunction with monthly hydrographic data from a nearby site in the Sargasso Sea for the following reasons:

- Bi-weekly sediment traps collected by the Oceanic Flux Program (OFP) are a suitable resolution for capturing the average 2-3 week lifecycle of most planktonic foraminifera (Erez et al. 1991, Spero, 1998).
- Monthly hydrographic data collected by the nearby Bermuda Atlantic Time Series (BATS) can be used to resolve the chemical and physical controls on planktonic foraminifera fluxes/shell parameters/geochemistry observed in the sediment traps.
- The OFP and BATS are both located in the Sargasso Sea, which is part of the oligotrophic (nutrient-poor) subtropical gyre. As oligotrophic regions characterise 75% of all open-ocean environments, results from this study have potential global significance.

The OFP sediment traps are the longest running record of their kind and have continuously measured particle fluxes in the deep Sargasso Sea since they were established in 1978. OFP traps are moored at 3200m, 1500m and 500m, have a sampling aperture of 0.5 m² and are fitted with a honeycomb baffle of 25 mm diameter cells, with a rotating carousel allowing for multiple samples to be collected in a single deployment. More details of the trap design and its operation can be found in Honjo and Doherty (1988). Werner Deuser pioneered the use of coarser resolution bimonthly sediment traps in the Sargasso Sea to demonstrate deep-sea seasonality in organic carbon (Deuser and Ross, 1980), sediment (Deuser et al. 1981), particle
(Deuser, 1986, 1996) and foraminifera fluxes (Deuser and Ross, 1989). Sediment traps are particularly useful for capturing planktonic foraminifera flux because their shells are relatively large and usually reach the traps within days and mostly without significant lateral displacement (Takahashi and Bé, 1984), especially the deeper traps (3200m and 1500m as used in this study). This has made sediment-trap studies of foraminifera particularly useful in determining species' global seasonality from e.g. the Panama Basin (Thunell and Reynolds, 1984), NE Pacific (Sautter and Thunell, 1989), Northern Atlantic (Storz et al. 2009), SW Pacific, Southern Ocean (King and Howard, 2005) to name just a few (please see Žarić et al. 2005 and Jonkers and Kucera 2015 for a full global sediment trap review). The ability of sediment traps to effectively capture seasonality of surface ocean fluxes without significant lateral contamination means that trap data can be directly compared to surface hydrography. For instance, the OFP traps are conveniently located near to the long running BATS hydrographic station, which has collected temperature and salinity measurements since 1955, and seawater nutrient, oxygen content, pigment and ocean chemistry data since 1989. The Bermuda Testbed Mooring site is also located next to the BATS site, collecting hourly meteorological measurements of wind speed, direction and light intensity. This makes our Bermuda study site an ideal location with which to address the research aims and questions posed in the next section.

1.5 Research aims

I will use the combination of high-resolution sediment trap time series samples in and hydrographic data to analyse the reliability of biogeochemical proxies in planktonic foraminifera and apply these to further understand how recent ocean acidification has affected their shell calcification. The research questions are as follows:
Chapter 1: Introduction

1. What physical/chemical/biological variables primarily control the species flux and growth of planktonic foraminifera? Chapter 2 focuses on seasonal and interannual changes in the total flux of planktonic foraminifera over two, 2.5-year periods in context with the physical and chemical changes in the water column. Species flux will also help evaluate the presence of 'optimum growth' conditions.


2. What controls the shell parameters and calcification of planktonic foraminifera? Chapter 3 establishes shell thickness/size relationships with shell flux (Salmon et al. 2015) and depth-adjusted hydrographic data to determine dominant environmental and growth controls on shell calcification. I digitally measure individual shells to effectively size-normalise and obtain accurate estimates of shell thicknesses and hence calcification.

Chapter 3, Paper 2: Salmon, K.H., Anand, P., Sexton, P., Bijma, J., Conte, M., Controls on shell parameters of modern planktonic foraminifera, to be submitted to Palaeoceanography.

3. What controls the trace element (and stable isotope) composition of planktonic foraminifera? Chapter 4 establishes relationships between various trace element concentrations in planktonic foraminifera with measured physical and chemical changes in the water column, and defines the importance of growth (size/flux) and calcification processes in the incorporation of trace element concentrations in planktonic foraminiferal calcite.
Chapter 4, Paper 3: Salmon, K.H., Anand, P., Sexton, P., Bijma, J., Conte, M.,
Testing the reliability of trace element proxies in planktonic foraminifera, to be

4. How has the calcification of planktonic foraminifera been affected by
anthropogenic ocean acidification? Chapter 5 compares shell calcification changes
observed in modern planktonic foraminifera from Chapter 3, with their pre-industrial equivalents collected in 1875 (Challenger Expedition) before the advent
of anthropogenic ocean acidification.

Chapter 5, Paper 4: Salmon, K.H., Anand, P., Sexton, P., Conte, M., Inhibition of
calcification in subtropical planktic foraminifera during the industrial era, submitted
to Nature Geoscience (June 2015).

By analysing species flux, shell size/thickness, stable isotope and trace element
geochemistry from bi-weekly, seasonally-resolved, pristine sediment trap samples, this
study will provide a unique ecological perspective on the interactions and processes which
control multiple biogeochemical proxies in planktonic foraminifera. A better understanding
of the ecological effects on the reliability of these proxies will aid palaeoceanographers in
the selection of suitable foraminiferal-based proxies with which to interpret past records of
environmental change.
Ada Lovelace (1815–1852)

"Understand well as I may, my comprehension can only be an infinitesimal fraction of all I want to understand."
2. Upper ocean mixing controls the seasonality of planktonic foraminifer fluxes and associated strength of the carbonate pump in the oligotrophic North Atlantic

This Chapter has been published as the following manuscript (January, 2015):

2.1 Abstract

Oligotrophic regions represent up to 75% of Earth's open-ocean environments. They are thus areas of major importance in understanding the plankton community dynamics and biogeochemical fluxes. Here we present fluxes of total planktonic foraminifera and eleven planktonic foraminifer species measured at the Oceanic Flux Program (OFP) time series site in the oligotrophic Sargasso Sea, subtropical western North Atlantic Ocean. Foraminifera flux was measured at 1500 m water depth, over two ~2.5 year intervals, 1998-2000 and 2007-2010. We find that foraminifera flux was closely correlated with total mass flux, carbonate and organic carbon fluxes. We show that the planktonic foraminifera flux increases approximately five-fold during the winter-spring, contributing up to ~40% of the total carbonate flux. This was primarily driven by increased fluxes of deeper dwelling glororotaliid species, which contributed up to 90% of the foraminiferal-derived carbonate during late winter-early spring. Interannual variability in total foraminifera flux, and in particular fluxes of the deep dwelling species (*Globorotalia truncatulinoides*, *Globorotalia hirsuta* and *Globorotalia inflata*), was related to differences in seasonal mixed layer dynamics affecting the strength of the spring phytoplankton bloom and export flux, and by the passage of mesoscale eddies. As these heavily calcified, dense carbonate tests of deeper dwelling species (3 times denser than surface dwellers) have greater sinking rates, this implies a high seasonality of the biological carbonate pump in oligotrophic oceanic
regions. Our data suggest that climate cycles, such as the North Atlantic Oscillation, which modulates nutrient supply into the euphotic zone and the strength of the spring bloom, may also in turn modulate the production and flux of these heavily calcified deep-dwelling foraminifera by increasing their food supply, thereby intensifying the biological carbonate pump.

2.2 Introduction

Planktonic foraminifera (PF) comprise 23-56% of the total open marine calcite flux and thus exert an important control on global carbon cycling (Schiebel, 2002). They are used extensively in palaeoceanographic and palaeoclimatic reconstructions via utilisation of their species abundance and assemblage composition (e.g., Lutz, 2011; Sexton and Norris, 2011), geochemical signatures (e.g., Zeebe et al. 2008), shell mass (e.g., Barker and Elderfield, 2002) and in evolutionary and biogeographic studies (e.g. Sexton and Norris, 2008). However, gaps remain in our understanding of the controls on their spatial and temporal distribution in the upper water column. Following the early 1980s when sea surface temperatures (SSTs) were thought to dominantly control PF distributions and abundance (CLIMAP project members, 1994), a number of other environmental parameters have also been shown to exert influence on the distribution and abundance of PF, such as salinity (Kuroyanagi and Kawahata, 2004), productivity, nutrient availability (Schiebel, 2002, Northcote et al. 2005, Žarić et al. 2005; Storz et al. 2009; Sexton and Norris, 2011) and water column stability (Hemleben et al. 1989, Lohmann and Schweitzer 1990, King and Howard, 2003). It is thus imperative to better understand the environmental factors controlling modern-day PF abundance in order to produce accurate interpretations of palaeorecords based on PF assemblages.

The response of PF flux and species composition to environmental and/or oceanographic factors have been studied using plankton tow materials which can give information about living populations' species distribution and depth habitats within the upper ocean (Tolderlund and Be, 1971, Fairbanks et al., 1980; Schiebel 2002). However, temporal
resolution is often limited when using plankton tows. The continuous time series records provided by sediment-traps allow a more complete understanding of the seasonal and interannual changes in PF flux and can aid in integrating living assemblages with the sedimentary record.

Earlier studies of planktonic foraminifer flux off Bermuda at the Seasonal Changes in Foraminifera Flux (SCIFF) site (Figure 2.1) (Deuser et al. 1981, Hemleben et al. 1985, Deuser 1987, Deuser and Ross 1989) were based on a bi-monthly sampling interval and provide a general description of foraminifera flux, species composition and seasonality. These studies found that PF >125μm comprise on average 22% of the total calcium carbonate flux in the Sargasso Sea (Deuser and Ross 1989), although this average underestimates the importance of the PF flux contribution during different seasons. Here we utilise a higher resolution bi-weekly sediment trap time series from the Oceanic Flux Program (OFP), ideal for studying the detailed response of PF species flux to physical oceanographic changes because PF species lifespan is approximately 2-3 weeks (Spero, 1998, Erez et al. 1991). These samples also benefit from the availability of upper ocean hydrographic and biogeochemical data collected at the nearby Bermuda Atlantic Time Series (BATS) site, as well as remote sensing data, which allows us to evaluate the environmental factors that control the total foraminifer flux as well as the response of individual species flux. Furthermore, we assess the relative contribution of PF flux to regional carbonate export and explore the implications of our findings for carbonate cycling in the oligotrophic North Atlantic.
2.3 Oceanographic Setting

The Sargasso Sea is located within the North Atlantic gyre, which is characterised by high temperatures and salinities, and weak, variable surface currents (Lomas et al. 2013 and references therein). The OFP and BATS sites are situated in a transition region between the northern eutrophic waters and the relatively oligotrophic subtropical convergence zone in the south (Steinberg et al. 2001 and references therein). Subtropical Mode Water (STMW) forms on the fringes, north of the gyre, owing to convective deep winter mixing and entrainment of nutrients and is characterized by temperatures of 17.8-18.4°C and salinities of ~36.5 +/- 0.05 (Bates et al. 2002), typically occurring between ~250-400 m water depth (Bates, 2007).
The hydrography and biogeochemistry of the area have been summarized by Michaels and Knap (1996), Steinberg et al. (2001), Lomas et al. (2013) and references therein. In the absence of large changes in salinity, the 10°C seasonal change in surface temperatures driven by solar insolation, controls the shoaling and erosion of the mixed layer, which reaches a maximum of 250-400m in late winter, increasing vertical mixing and entraining nutrient-rich waters. The depth of mixing determines the strength of seasonal particulate flux, nutrient concentrations and primary production during the subsequent spring bloom (Michaels and Knap, 1996, Steinberg et al., 2001). With the onset of seasonal stratification in late February-March, a spring bloom develops when phytoplankton biomass and particulate organic carbon standing stocks are maximal. As seasonal stratification intensifies, a nutrient-depleted, shallow surface mixed layer develops which is underlain by a subsurface chlorophyll maximum at approximately 80-100m depth. Strong stratification in summer and autumn results in low vertical mixing that limits nutrient availability and primary production. Seasonal cooling in late autumn results in erosion and gradual deepening of the mixed layer, with renewed nutrient entrainment into the euphotic zone and an increase in primary production. Mesoscale physical variability in this area is the dominant method of nutrient transport (McGillicuddy et al., 1998). In particular, passage of cyclonic and mode water eddies may lead to nutrient entrainment which generates short-lived phytoplankton blooms and community restructuring (Wiebe and Joyce, 1992, Olaizola et al., 1993, McNeil et al., 1999, Letelier et al., 2000, Seki et al., 2001, Sweeny et al., 2003) which could, in turn, impact higher trophic levels such as planktonic foraminifera. In addition, these blooms often result in short-lived, episodic periods of enhanced export fluxes of labile organic material to depth (Conte et al. 1998, 2003, 2014).
Chapter 2: Planktonic foraminifera fluxes

2.4 Materials and methods

2.4.1 The OFP Sediment trap time-series
The OFP mooring is located at 31° 50'N, 64° 10'W, about 55 km southeast of Bermuda at 4200m water depth (Figure 2.1). Three Mark VII Parflux sediment traps (McLane Labs, Falmouth, MA) are deployed at depths of 500 m, 1500 m and 3200 m. The traps (0.5 m² surface area) are programmed to collect a continuous bi-weekly time-series of the particle flux. Collected samples were processed according to Conte et al. (2001) and split into < 125 µm, 125-500 µm, 500-1000 µm and >1000 µm size fractions. We analyzed foraminifera in the 125-500 µm and 500-1000 µm size fractions of 1500m trap samples collected during two time periods: 1998-2000 and 2008-2010 (109 samples total). We selected the two equivalent 2.5 year intervals a decade apart to generate a bi-weekly resolved time-series which would enable assessment of seasonality as well as interannual variability. Our analyses focused on eleven species that fall within three general groupings: i) surface dwelling species living within the upper 50 m water column (Globigerinoides ruber var. white/pink, Globigerinella siphonifera, Globigerinoides sacculifer), ii) intermediate dwelling species living in the ~50-200 m depth range (Orbulina universa, Globigerinoides conglobatus, Neogloboquadrina dutertrei, Puleinatina obliquiloculata) and iii) deep dwelling species (or species that are thought to calcify over a large depth range) living in the ~100-800 m depth range (Globorotalia inflata, Globorotalia crassaformis, Globorotalia truncatulinoides, Globorotalia hirsuta). Our assignments of the depth habitats were based on measured species depth distributions and/or inferred distributions based on oxygen isotopic composition (Fairbanks et al., 1980, Anand et al., 2003). The temporal offset between the foraminiferal species fluxes reaching the trap at 1500m depth versus the timing of these species' growth in overlying waters will vary depending on habitat depths and individual species' sinking rates (Takahashi and Bé, 1984). A surface-dwelling G. ruber living at 25 m depth may sink at ~198 m day⁻¹, taking ~7 days to reach the 1500 m trap, whereas a more heavily calcified deeper-dwelling
species such as *G. inflata* may sink ~504 m day⁻¹, taking only ~3 days to reach the 1500 m trap. These fast sinking rates are much shorter than the typical lifespans of PF and are thus not anticipated to cause any offset between the hydrographic and sediment trap flux data (Honjo and Manganini, 1993).

On average, ~440 tests were counted in each sample fraction. To generate the flux data, counts of total and individual foraminifera species in the sample aliquots for each size fraction was converted to total counts per sample fraction and then the totals for the two fractions were combined (i.e. total planktonic foraminifera between 125-1000 µm in size). Total counts were then scaled for the processing split (60%) and converted to flux (tests m⁻² d⁻¹).

### 2.4.2 BATS and remote sensing data

The BATS site (31°40'N, 64°10'W) is located just south of the OFP mooring (Figure 2.1). Monthly hydrographic and biogeochemical data collected by the BATS time-series was obtained from the BATS website ([http://bats.bios.edu](http://bats.bios.edu)). Mixed layer depth (MLD) was available from Lomas et al. (2013) and was calculated from CTD profiles using the variable sigma-t criterion equivalent to a 0.2°C temperature change (Sprintall and Tomczak, 1992). The mesoscale eddy field was assessed using interpolated data on sea surface anomaly available from the CCAR Global Historical Gridded SSH Data Viewer ([http://eddy.colorado.edu/ccar/ssh/hist_global_grid_viewer](http://eddy.colorado.edu/ccar/ssh/hist_global_grid_viewer)).
2.5 Total planktonic foraminiferal fluxes

2.5.1 In relation to other mass fluxes

The seasonal cycle and interannual variability of the PF flux at 1500m depth is highly correlated with that of the total mass, carbonate and organic carbon fluxes. All fluxes are strongly characterized by an abrupt spring maximum during February-April, which varies significantly on an interannual basis (Figure 2.2). For example, the spring PF flux peak ranged from a low of 400 tests m\(^{-2}\) day\(^{-1}\) in 2008, coinciding with minimal spring mass fluxes, to a high of 900 tests m\(^{-2}\) day\(^{-1}\) in 2009, coinciding with an extreme peak in spring mass fluxes. All fluxes typically drop to a minimum over the summer months (May-August) and remain low until the following spring bloom. During these minima, the PF flux generally amounts to <200 tests/m\(^2\)/day. In some years (e.g. 2009 and, to a lesser extent, 2008), the PF flux displays a smaller, but distinct second peak in the months September-October. This secondary autumn peak can also be seen in the mass flux and carbonate flux in 2009 but is absent in the organic carbon flux. Over the entire record, the correlation between PF flux and mass, carbonate and organic carbon flux is 0.65, 0.64 and 0.55, respectively.
2.5.2 Relative to upper ocean hydrography

In Figure 2.3 we compare interannual variations in bi-weekly resolved total PF flux to ~monthly resolved changes in key upper ocean hydrographic parameters, measured at the BATS site. PF flux exhibits an inverse relationship with seasonal variations in sea surface temperatures (SST) and reaches a maximum when SST is coolest in January-March (Figure 2.3a). Of note, is the particularly large and prolonged PF bloom in 2010, which coincided with a cyclonic eddy that passed through the area causing the lowest SSTs on record for this site ~18.9°C (Figure 2.3a-b).
Figure 2.3. Temporal changes in environmental parameters measured at the BATS site in relation to total planktic foraminiferal flux in the 1500m OFP trap (thin, black line) a) Sea surface temperature (0-25 m), b) Sea level height anomaly; grey bars indicate periods when productive cyclonic eddies influenced the site, c) Mixed layer depth, d) Chlorophyll a concentration (0-25 m average) e) Average organic carbon flux at 200 m
Sea level anomaly (SLA) provides information about eddies passing through the area (Figure 2.3b). A negative anomaly is associated with cyclonic eddies and a positive anomaly associated with anticyclonic and mode water eddies. The SLA data show that the particularly high and prolonged PF fluxes, total mass flux and organic carbon flux in spring 2009 and 2010 coincided with the passage of cold, cyclonic eddies (Figure 2.2), which enhance nutrient upwelling into the euphotic zone.

The annual and interannual PF flux is in phase with the deepening and shoaling of the mixed layer depth (MLD) (Figure 2.3c) and with chlorophyll a concentrations (Figure 2.3d). The seasonal PF flux maximum coincides with the chlorophyll a maximum (which is used here as a proxy for the spring phytoplankton bloom) and the organic carbon flux from 200m, which represents organic carbon export from surface productivity (Figure 2.3e), and the deepest MLD during February-March. During April-May, the MLD shoals back towards the surface coinciding with decreasing chlorophyll a concentrations and PF flux. The strong correlations between the seasonality in PF flux and that of primary production and export is demonstrated by the regressions between total PF flux and chlorophyll a concentration (Figure 2.4a) and the 1500m mass flux (Figure 2.4b). During the winter-spring period the magnitude of PF flux generally follows the evolution in MLD and is maximal when the MLD is maximal (Figure 2.4c). However, when the mixed layer depth shoals to <80 m during the low productivity period in late spring and summer, this correlation is not significant (Figure. 2.4d).
2.6 Planktonic foraminifera species fluxes

In general, all planktonic foraminifera, and especially deeper dwelling species, show strong, consistent seasonal variance (Figures 2.5-2.7).
Chapter 2: Planktonic foraminifera fluxes

Figure 2.5. Temporal changes in surface dwelling planktic foraminifera fluxes in the 1500m trap with changes in sea surface temperature (0-25 m) shown in the dashed black line for reference. The approximate depth habitat (Anand et al. 2003) is shown on figures.
Figure 2.6. Temporal changes in intermediate dwelling planktonic foraminifera fluxes in the 1500 m trap with changes in sea surface temperature (0-25 m) for reference. The approximate depth habitat (Anand et al. 2003) is shown on figures.
Figure 2.7. Temporal changes in deeper dwelling planktonic foraminifera fluxes in the 1500 m trap with changes in sea surface temperature (0-25 m) for reference. The approximate depth habitat (Anand et al. 2003) is shown on figures. Graphs are ordered according to seasonal succession.

Our results demonstrate a clear depth progression towards more pronounced seasonality in the deeper species, compared to a larger intra-seasonal variability in the surface and intermediate dwellers. In addition, the deep dwelling PF species exhibit repeatable species successions throughout the winter and early spring (Figure 2.8, Table 2.1). Figure 2.8 shows that *Globorotalia truncatulinoides* dominates the flux of deeper dwellers, and thrives each December, reaching a maximum during January. *G. truncatulinoides* is then followed by *G. hirsuta*, *G. crassaformis*, and *G. inflata* which all peak between March and April.
Chapter 2: Planktonic foraminifera fluxes

Figure 2.8. Seasonal succession for deeper dwelling species averaged over six spring blooms (1998, 1999, 2000, 2008, 2009, 2010) from the 1500 m trap. *G. truncatulinoides*, *G. hirsuta*, *G. inflata* appear on the left axis and *G. crassaformis* is on the right axis.

*G. truncatulinoides* displays large interannual variability (Table 2.1), ranging from lows of ~4000 tests/m²/year in 2009-2010 to highs of up to ~14 000 tests/m²/year in 1999-2000 (Figure 2.6). The remaining deeper dwellers (*Globorotalia hirsuta*, *Globorotalia inflata*, *Globorotalia crassaformis*) also vary on an interannual basis. Figure 2.7 and Table 2.1 show that the largest fluxes of deeper dwelling species occurred during the winter/spring of 1999-2000 and 2008-2009. Using shell weights from this study averaged with shell weights (125-1000um) measured by Deuser, (1987) and Deuser and Ross, (1989), we estimate that PF flux contributes up to ~40% of the total carbonate flux during winter-spring but <10% during summer (Figure 2.9a). Deeper dwelling species account for 60-90% of PF carbonate flux (Figure 2.9b) and up to 37.5% of the total carbonate flux (e.g. during the winter-spring of 2000 (Figure 2.9c).
Figure 2.9. a) The relative contribution of total PF to total carbonate flux b) The relative contribution of deeper dwelling planktonic foraminifera (G. hirsuta, G. truncatulinoides, G. crassaformis, G. inflata) to the total planktonic foraminiferal carbonate flux c) The relative contribution of total deeper dwellers (G. hirsuta, G. truncatulinoides, G. crassaformis, G. inflata) to the total carbonate flux. All graphs show four full years 1998-99, 1999-00, 2008-09 and 2009-10.
## Table 2.1. Annual fluxes for planktonic foraminifera species at 1500 m depth in 1998-1999, 1999-2000, 2008-2009 and 2009-2010 and the four-year averages. Fluxes were calculated from the sum of biweekly averages between July-June for each year and converted to tests m\(^{-2}\) yr\(^{-1}\). Species are listed according to their estimated depth habitats.

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<tr>
<td><em>G. ruber</em> (pink)</td>
<td>July-Sept</td>
<td>2524</td>
<td>1978</td>
<td>1576</td>
<td>2122</td>
<td>2050</td>
<td>1450</td>
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<td><em>G. ruber</em> (white)</td>
<td>Sept-Oct</td>
<td>16197</td>
<td>19633</td>
<td>13917</td>
<td>18719</td>
<td>17117</td>
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<tr>
<td><em>G. sacculifer</em></td>
<td>Oct(^1), March(^2)</td>
<td>256</td>
<td>292</td>
<td>1007</td>
<td>348</td>
<td>1903</td>
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<td>21903</td>
<td>16500</td>
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<td>17346</td>
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<tr>
<td><em>G. siphonifera</em></td>
<td>*</td>
<td>6101</td>
<td>3182</td>
<td>2231</td>
<td>2833</td>
<td>3587</td>
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<tr>
<td><em>O. universa</em></td>
<td>April-May(^1), Oct-Nov(^2)</td>
<td>1429</td>
<td>694</td>
<td>1056</td>
<td>2250</td>
<td>1357</td>
<td></td>
</tr>
<tr>
<td><em>G. conglobatus</em></td>
<td>Nov</td>
<td>277</td>
<td>180</td>
<td>0</td>
<td>4</td>
<td>115</td>
<td>300</td>
</tr>
<tr>
<td><em>N. dutertrei</em></td>
<td>March-April(^1), Nov-Dec(^2)</td>
<td>1290</td>
<td>185</td>
<td>471</td>
<td>839</td>
<td>696</td>
<td>876</td>
</tr>
<tr>
<td><em>P. obliquiloculata</em></td>
<td>Dec-March</td>
<td>398</td>
<td>205</td>
<td>708</td>
<td>352</td>
<td>416</td>
<td>762</td>
</tr>
<tr>
<td><strong>Intermediate Totals</strong></td>
<td></td>
<td>9495</td>
<td>4446</td>
<td>4466</td>
<td>6278</td>
<td>6171</td>
<td></td>
</tr>
<tr>
<td><strong>Deep dwellers:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. truncatulinoides</em></td>
<td>Jan-Feb</td>
<td>5248</td>
<td>13796</td>
<td>9517</td>
<td>4031</td>
<td>8148</td>
<td>3420</td>
</tr>
<tr>
<td><em>G. hirsuta</em></td>
<td>Feb-March</td>
<td>1784</td>
<td>9888</td>
<td>3859</td>
<td>2770</td>
<td>4575</td>
<td>1520</td>
</tr>
<tr>
<td><em>G. crassaformis</em></td>
<td>Feb-March</td>
<td>26</td>
<td>100</td>
<td>122</td>
<td>139</td>
<td>97</td>
<td>192</td>
</tr>
<tr>
<td><em>G. inflata</em></td>
<td>March-April</td>
<td>844</td>
<td>995</td>
<td>1652</td>
<td>1869</td>
<td>1340</td>
<td>1270</td>
</tr>
<tr>
<td><strong>Deep Totals</strong></td>
<td></td>
<td>7902</td>
<td>24779</td>
<td>15150</td>
<td>8809</td>
<td>14160</td>
<td>5402</td>
</tr>
<tr>
<td><strong>Other species</strong></td>
<td></td>
<td>51442</td>
<td>43704</td>
<td>43172</td>
<td>70446</td>
<td>51191</td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td>87816</td>
<td>94831</td>
<td>79289</td>
<td>106722</td>
<td>92165</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Primary peak, \(^2\) Secondary peak, \(^3\) Averages from Deuser and Ross, 1989, * This species has low seasonality
2.7 Discussion

The controls on PF flux in the Sargasso Sea was first introduced by Bé, (1960) and later developed by Tolderlund and Bé, (1971) who suggested that PF flux is dominantly controlled by the availability of their food phytoplankton. Thus, the environmental factors controlling PF flux should be closely aligned with the factors controlling phytoplankton productivity and export flux.

2.7.1 Depth of the mixed layer

Previous studies suggest that increased chlorophyll concentrations and larger phytoplankton abundances occur when the MLD deepens (Townsend et al. 1994, Waniek, 2003, Nelson et al. 2004) and the amplitude and timing of MLD deepening determines the size of the following spring bloom (Menzel and Ryther, 1961, Michaels et al. 1994). Here, we also observe a simultaneous seasonal peak in chlorophyll a and maximum depth of the MLD, as observed by previous studies at BATS (Steinberg et al. 2001, Cianca et al. 2012), the timing and amplitude of which coincides with the maximum PF flux (Figure 2.3c, d). Similarly, seasonal changes in mixed layer depth are closely associated with changes in foraminifer production (Thunell and Reynolds, 1984, Sautter and Thunell, 1989, Pujol and Vergnaud Grazzini 1995, Schmucker and Sciebel 2002) and chlorophyll a concentrations (King and Howard, 2003, 2005) in other ocean basins. Siegel et al. (2002) proposed that south of 40°N, the initiation and extent of the spring bloom is dominantly limited by nutrients, and this is supported by the simultaneous increase in phytoplankton concentrations with mixing depth at BATS (Treusch et al. 2012). Vertical mixing in late winter and spring distributes nutrients into the euphotic zone to support the spring phytoplankton bloom, causing the consequent seasonal peak in export fluxes of organic carbon, to fuel symbiont-barren foraminifera production (Figure. 2.2d). In contrast, no correlation exists between PF flux and MLD during the late spring to autumn when the mixed layer fails to penetrate the minimum depth of the deep chlorophyll maximum layer (~80m), where many species of planktonic foraminifera reside in association with other
zooplankton and algal cells (Fairbanks and Wiebe, 1980) (Figure 2.4d). This is also the depth of the nitricline where nitrate concentrations > 0.1 umol kg\(^{-1}\), (Sciebel et al. 2001).

The majority of the increased PF flux in the winter-spring is driven by increased fluxes of deeper dwelling species, in particular \textit{G. truncatulinoides} and \textit{G. hirsuta} (Figure, 2.9b). These species are symbiont-barren and rely on the flux of phytodetritus and other labile organic carbon as a food source from the spring phytoplankton bloom (Hemleben et al. 1989). The discrepancy in timing of peaks between the deeper dwelling species (Figure 2.8) is likely due to subtle changes in phytoplankton succession related to the species' diets (Deuser and Ross, 1989, Hemleben et al. 1989). Overall, the seasonal PF species succession is broadly similar to previous observations from 1959-63 and 1978-84 (Tolderlund and Bé, 1971, Deuser 1987, Deuser and Ross, 1989) which suggests that despite long-term environmental change, species seasonality have remained consistent over the past 50 years.

The correlation observed here between the seasonality in the PF flux, chlorophyll \text{a} concentration and mass flux at 1500m (Figure 2.4 a and b) clearly demonstrates that the seasonality of non symbiont-bearing foraminifera, such as the globorotaliids is controlled by phytoplankton production and the export flux of phytodetritus to depth. As these globorotaliids are up to three times denser than surface species (unpublished data), their sinking rates are significantly higher than those of other species. Thus, increased production by these species can accelerate the transfer of carbonate from surface to deep-ocean, thereby strengthening the carbonate pump.

In contrast, the surface-dwelling symbiont-bearing foraminifera have lifecycles which strongly benefit from stratified surface waters and shallow mixed layers in order to photosynthesise allowing them to succeed in low nutrient conditions (Hemleben et al. 1989). Surface dwellers generally calcify in late summer when sea surface temperatures are at a maximum and dinoflagellates are abundant (Tolderlund and Bé, 1971). We thus
conclude that the depth and structure of the mixed layer plays an important role in regulating PF species flux by controlling the abundance and timing of their food availability throughout the seasonal cycle.

2.7.2 MLD deepening and shoaling rates

Current models based on the light-limited higher latitudes (Waniek, 2003; Mao, Y., 2013-personal communication), suggest that if the MLD shoals early and slowly, the consequent bloom will be long and weak compared to if the MLD shoals late and quickly, which causes a short and sharp bloom. At our subtropical study site, the spring bloom is predominantly limited by nutrient input into the euphotic zone, which is determined by the depth of the mixed layer. Increased heat loss and wind stress leading to higher convective mixing during the winter months controls the rate of deepening of the mixed layer, which is strongly correlated to the maximum MLD reached \( r^2 = 0.88 \) (Figure 2.10a). Years with faster deepening rates have deeper mixed layers and hence larger spring blooms (e.g. winter 2009), whereas slow deepening rates cause shallower mixed layers and smaller spring blooms. There is also some evidence that light-limitation could be a secondary control on the peak productivity of the spring bloom at this site (Dutkiewicz et al. 2001, Lomas et al. 2009, Cianca et al. 2012) as the euphotic zone extends to ~100m (Steinberg et al. 2001) and a faster shoaling rate during the spring could control could concentrate the food available for symbiotic-foraminifera in the euphotic zone, resulting in a larger PF flux.

To test whether the rates of mixed layer deepening in early winter and of shoaling in spring affect the PF flux, we computed a mixed layer dynamics index, \( \frac{D_r}{S_r} \), which is the ratio of the rate of deepening to the rate of shoaling and compared this to the integrated PF flux (Table 2.2). The \( \frac{D_r}{S_r} \) ratio never exceeds 1, indicating that the shoaling rate always exceeds the deepening rate. For all the years studied, there is a strong inverse relationship between the integrated PF flux over the duration of spring bloom, and the \( \frac{D_r}{S_r} \) ratio (Figure 2.10b, \( r^2 = 0.93 \)). This relationship is also present in the maximum in
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chlorophyll $a$ concentration and the $D_\alpha/S_\alpha$ ratio (Figure 2.10c, $r^2 = 0.76$). This correlation indicates that when the MLD shoals more quickly during spring stratification (lower $D_\alpha/S_\alpha$ ratio), the chlorophyll $a$ concentrations and PF flux are higher, as supported by a strong correlation ($r^2 = 0.87$) between shoaling rate and integrated PF flux (Figure 2.10d).
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Figure 2.10. a) Correlation between the maximum mixed layer depth and deepening rate of the mixed layer for years 1995-2011. Correlation between the deepening:shoaling rate (D/S) ratio of the mixed layer depth for all years studied excluding 2000 and b) Integrated PF flux during the spring blooms which ranged from Dec-May c) maximum chlorophyll a concentrations in the surface ocean during the spring bloom for all years studied, excluding the anomalous year 2010 in parentheses d) Correlation between the shoaling rate and integrated flux of total PF over the spring bloom period which ranged from Dec-May. Diamonds indicate years with eddy influence 2009 and diamond with parentheses = 2010. Round points are years without eddy influence.
**Table 2.2.** Mixed layer depth and mean rates of mixed layer (ML) deepening and shoaling. The \( \text{D}_{\text{Sr}} / \text{Sr} \) ratio is a derived value calculated from the rate of ML deepening divided by the rate of ML shoaling (see text). The winter-spring PF flux represents the PF flux integrated over the whole bloom, which varied interannually in length but ranged from Dec-May. Years highlighted bold indicate when a cyclonic eddy was present during the spring bloom period.

<table>
<thead>
<tr>
<th>Year</th>
<th>MLD max (m)</th>
<th>ML Deepening Rate (m day(^{-1}))</th>
<th>ML Shoaling Rate (m day(^{-1}))</th>
<th>( \text{D}_{\text{Sr}} / \text{Sr} ) ratio (m)</th>
<th>Maximum PF flux (tests m(^{-2}) day(^{-1}))</th>
<th>Integrated winter-spring PF flux (tests m(^{-2}) day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997-1998</td>
<td>235</td>
<td>0.93</td>
<td>1.91</td>
<td>0.49</td>
<td>641</td>
<td>28</td>
</tr>
<tr>
<td>1998-1999</td>
<td>222</td>
<td>0.78</td>
<td>7.78</td>
<td>0.10</td>
<td>816</td>
<td>41</td>
</tr>
<tr>
<td>1999-2000</td>
<td>197</td>
<td>0.63</td>
<td>Data missing</td>
<td>-</td>
<td>761</td>
<td>30</td>
</tr>
<tr>
<td>2007-2008</td>
<td>130</td>
<td>0.55</td>
<td>0.75</td>
<td>0.73</td>
<td>385</td>
<td>17</td>
</tr>
<tr>
<td><strong>2008-2009</strong></td>
<td><strong>198</strong></td>
<td><strong>0.95</strong></td>
<td><strong>2.21</strong></td>
<td><strong>0.43</strong></td>
<td><strong>946</strong></td>
<td><strong>28</strong></td>
</tr>
<tr>
<td><strong>2009-2010</strong></td>
<td><strong>464</strong></td>
<td><strong>1.76</strong></td>
<td><strong>3.82</strong></td>
<td><strong>0.46</strong></td>
<td><strong>815</strong></td>
<td><strong>32</strong></td>
</tr>
</tbody>
</table>
Years where the shoaling rate is twice as quick as the deepening rate (e.g. winters 1997, 2008, and 2009) have average D$_i$/S$_r$ ratios, average-length blooms and PF flux (~30 tests/m$^2$/day, Table 2.2). Years with comparatively equal rates of shoaling and deepening (e.g. winter 2007) have larger D$_i$/S$_r$ ratios, longer and slower blooms with shallower MLDs and small PF fluxes. Years when the shoaling rate is much quicker than deepening rate e.g. winter 1999 have the smallest D$_i$/S$_r$ ratios and shorter, sharper blooms with greater numbers of intermediate thermocline dwelling species such as *N. dutertrei*, *P. obliquiloculata*, *G. siphonifera*, suggesting that when the rate of shoaling is higher the seasonal thermocline is nearer to the surface for longer, which is beneficial for these symbiont-bearing and symbiont-facultative species. The PF fluxes were large (and prolonged) respectively in winter 2008-09 and 2009-10 despite having average D$_i$/S$_r$ ratios but were probably enhanced by additional factors discussed in the next section.

### 2.7.3 Eddies

The negative sea level anomalies in spring of 2009 and 2010 indicate that the large (and in 2010 prolonged) PF fluxes in these years were clearly associated with the passage of cyclonic eddies (Figure 2.3b). Eddy pumping of nitrate into the euphotic zone has been shown to significantly increase new production (Oschlies and Garçon, 1998, Oschlies, 2002). Cianca et al. (2007) estimate that eddy pumping contributes ~50% of the nutrient input into the euphotic zone in the Sargasso Sea. Studies at the BATS site have demonstrated the influence of cyclonic and mode water eddies in promoting phytoplankton blooms and increased secondary production (Eden et al., 2009, McGillicuddy et al. 2007, Goldthwait and Steinberg, 2008, McGillicuddy et al., 1999, Sweeney et al., 2003, Lomas et al. 2013, Cianca et al. 2012) and therefore affecting PF food availability and quality (Schmuker and Schiebel, 2002). Previous studies have found higher fluxes of certain PF species such as *Globigerinita glutinata* associated with cyclonic eddy structures in the Caribbean Sea (Schmuker and Schiebel, 2002) and North Atlantic (Beckman et al. 1987), also in conjunction with upwelling frontal regions in the Mexican Pacific (Machain-Castillo et al. 2008) and deep mixed layers during winter in the
Mediterranean (Pujol and Vergnaud Grazzini, 1995). Here we observe a similar response during the passage of a cyclonic eddy in spring 2009, particularly for deeper dwelling species. In fact, the largest PF flux observed over the entire record was associated with this eddy passage, even though the maximum MLD and D$_{15}$/S, were modest (Table 2.2). Similarly, the mass and organic carbon flux measured during passage of this eddy (Figure 2.2b-d) were the highest fluxes measured over the last 25 years of the OFP time-series, indicating that the conditions in this eddy promoted an extremely large export flux to fuel the production of deep dwelling foraminifera species such as *G. truncatulinoides*, *G. hirsuta*, and especially *G. inflata* which all experienced higher seasonal fluxes in 2009 (Figure 2.7).

This observation is consistent with an exceptionally large increase in the flux of *G. truncatulinoides* (> 600 tests m$^{-2}$ day$^{-1}$) seen at the OFP traps during the spring of 2007, which was also influenced by the passage of a productive cyclonic eddy (Fang et al. 2010, Conte et al. 2014). Both the 2007 and 2009 eddies occurred between January-March during the seasonal flux of the deeper dwellers (Figure 2.7), underscoring the importance of the timing of eddy passage in enhancing PF flux. The influence of eddies here is similar to observations from the Eastern Mediterranean where increased numbers of grazing species such as *G. truncatulinoides* and *G. inflata*, have been found in association with eddy structures and deep mixed layers (Pujol and Vergnaud Grazzini, 1995). These findings suggest that productive cyclonic eddies, when co-occurring with deep MLDs, act to enhance the existing seasonal abundance of deeper dwelling species through mixing of the water column, which aids their annual reproductive migration in addition to increasing food supply.

Along with the timing of the eddy passage, our observations also suggest that the PF flux response is dependent on whether the eddy is intensifying or weakening. For instance, both cyclonic eddies in 2009 and 2010 intensified over the spring bloom (Figure 2.3b) eliciting a large biological response indicated by elevated subsurface Chl-a concentrations.
and increased PF flux. In contrast, the cyclonic eddy in winter 2007-08 was weakening over the spring bloom and therefore elicited no PF flux response. Recent studies have found that eddies which are a minimum of 1-2 months in duration are more likely to induce a larger biological response (Mouriño-Carballido and McGillicuddy, 2006, Sweeny et al. 2003). Our observations also suggest that eddies need to be present for at least a month to elicit responses in the flux of PF which have minimum lifecycles of two weeks. For instance, in winter 1998-99 a cyclonic eddy passed over the sediment trap site in only one month and elicited no biological response, compared to cyclonic eddies in 2009 and 2010, which both remained over the site for a minimum of 2-3 months and elicited large biological responses (Figure 2.3b). These findings suggest that cyclonic eddies which intensify over the spring bloom and last for 1-3 months can elicit a significant biological response and increased PF flux.
2.8 Implications

Our results show that environmental factors and mesoscale eddy variability play an important role in regulating the planktonic foraminifera fluxes, by regulating the MLD and consequent magnitude of the spring bloom and biological export flux.

An overarching climatological variable affecting this region especially is the North Atlantic Oscillation (NAO), which exerts a strong influence on air temperature, storminess, heat loss, winter mixed layer depth, and, therefore, nutrient injection into the upper ocean during the winter months (Bates, 2012, Bates and Hansell, 2004, Rodwell et al. 1999). Modelling studies have shown that when the NAO is in its low phase, i.e. negative NAO (e.g. winter 2010), there is increased heat loss that intensifies convective mixing and results in enhanced nutrient upwelling into the euphotic zone to support primary production (Oschlies, 2001). The NAO influence on upper ocean productivity and biogeochemical fluxes is demonstrated by the inverse correlation between the wintertime (NDJF) NAO index and the deep particulate nitrogen flux in the OFP traps over a thirty-year period (Conte and Weber, 2014) and increased primary productivity in negative wintertime NAO phases (Lomas et al. 2010). If convective mixing and nutrient entrainment into the euphotic zone is stronger during negative NAO years, this could serve to modulate PF flux, and therefore carbonate flux, on decadal timescales. When we compare PF fluxes covering a range of NAO indexes, from this study using the 1500m trap to the 3200m trap between 1978-84 (Deuser and Ross, 1989, Deuser, 1987), we find a weak inverse correlation between total PF flux and (DJFM) NAO index in-phase (not significant), but we do find a significant inverse correlation with a (DJFM) NAO with a 1-year lag ($p < 0.005$) (Figure 2.11). Cianca et al. (2012) showed that their correlation between winter NAO and total Chlorophyll a at BATS improved when applying a +1 year time lag, but still remained insignificant. They attributed this to variability in the subtropical mode water, which can laterally advect nutrients on interannual timescales (Palter et al. 2005, Patara et al. 2011). We acknowledge that additional longer-term data is needed to
test the mechanism behind this correlation, but our results suggest that changes in NAO status and/or mesoscale eddy frequency could significantly modulate planktonic foraminifera flux and export flux from the surface ocean on interannual timescales.

**Figure 2.11.** Annual integrated PF flux from this study (1500m trap, square symbols) and 1979-1984 (*3200m trap, round symbols, Deuser, 1987, Deuser and Ross, 1989) plotted against wintertime (DJFM) NAO index + 1 year lag. Annual fluxes from both trap depths are comparable. **Annual PF flux from 1978 (diamond symbol) was not included in the regression because it was an anomalously low flux year which could be explained by a shallow MLD and/or possibly the presence of an anticyclonic eddy (no data to test), which may have suppressed the spring bloom and hence PF flux as seen during 1994 at BATS (Lomas et al. 2013). NAO data available from http://www.cpc.ncep.noaa.gov/data/teledoc/nao.shtml

This study shows that the productivity of the dominant deep dwelling species *G. truncatulinoides* and *G. hirsuta* is especially responsive to interannual variability in overlying surface water conditions and especially to the transient high production/flux events that are associated with the passage of productive cyclonic eddies that coincide with their seasonal spring production peak. Our data show that deeper dwelling species can account for up to ~90% of the total PF carbonate flux, representing up to ~40% of the total carbonate flux during winter-spring at the OFP site. Changes in NAO status, which
modulates nutrient supply into the euphotic zone and the strength of the spring bloom, also may in turn modulate the production and flux of these heavily calcified deep-dwelling foraminifera by increasing their food supply, thereby intensifying the carbonate pump.

2.9 Conclusions

Our study demonstrates that the interannual variability in planktonic foraminifera flux can be linked to the MLD and the rate of deepening/shoaling of the mixed layer associated with nutrient injection into the euphotic zone. We find that higher PF fluxes coincide with deeper MLDs, especially when combined with cyclonic eddy-induced nutrient upwelling. In particular, the production of the dominant deep dwelling species *G. truncatulinoides* and *G. hirsuta* is shown to be particularly responsive to interannual variability in overlying surface water conditions and especially to the transient high production/flux events that are associated with productive cyclonic eddies. These species dominate the major late winter-early spring pulses of foraminifera and have higher sinking rates than surface dwelling species because they are up to three times denser (unpublished results). We suggest deeper-dwelling species strengthen the carbonate pump by accelerating the transfer of carbonate from surface to deep ocean and contribute up to 40% of the contemporaneous peak in total carbonate export fluxes. It follows that any increase in fluxes of these deep-dwellers arising from climate-induced changes in winter-spring mixed layer dynamics will also increase the average sinking rate of foraminiferal carbonate and intensify the overall carbonate pump. Our findings suggest that the North Atlantic Oscillation, via its influence on mixed layer depth, nutrient upwelling, phytoplankton production and export flux may also serve to modulate the foraminiferal component of the carbonate pump in the subtropical North Atlantic.

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**Contributions**

K. S. selected the sampling period, collected the data and interpreted results. P. A. conceived the project idea, and P.A. and P.F.S. secured funding. M. C. provided the sediment trap samples. All authors contributed their relevant expertise to constructing the manuscript.
Marie Skłodowska Curie (1867-1934)

"Nothing in life is to be feared; it is only to be understood"
Chapter 3: Shell parameters in modern planktonic foraminifera

3. Controls on shell parameters of modern planktonic foraminifera

This Chapter has been written to submit to *Paleoceanography*:

3.1 Abstract

Shells of planktonic foraminifera (PF) can contribute up to 80% of CaCO₃ globally to marine sediments and are important components of the marine carbon cycle, acting as both a source and sink of CO₂. It is therefore critical to determine which environmental variables dominantly control the PF calcification in order to understand how planktonic foraminifera will respond to anthropogenic-induced acidification and warming. In this study, we compare the area densities (a proxy for shell thickness and therefore calcification) of two depth-stratified PF species *Globigerinoides ruber* (p) and *Orbulina universa* from a bi-weekly sediment-trap time series, with their respective species fluxes and size measurements, and monthly measurements of [CO₃²⁻], temperature, and chlorophyll a concentrations in the Sargasso Sea. We employ multiple linear regression to determine that both temperature and [CO₃²⁻] explain most of the variability in the shell thickness and therefore calcification of these species ($r^2 = 0.62$), whilst water density describes the majority of variability in the test size ($r^2 = 0.78$), probably through regulation of buoyancy. Although we find no 'optimum growth condition' control (inferred from species flux and size) on shell thickness, *O. universa* shell thickness does increase with shell flux during a transient cyclonic eddy coincident with a prolonged subsurface chlorophyll a maximum. Sediment-trap area density-[CO₃²⁻] relationships are up to 4-times more sensitive than equivalent culture relationships caused by either genotypic variability between cultured and open-ocean *O. universa*, or the synergic effects of [CO₃²⁻].
Chapter 3: Shell parameters in modern planktonic foraminifera

and temperature together creating a greater net change in shell thickness and therefore calcification when compared to limited changes of these variables in culture experiments.

3.2 Introduction

The oceans have absorbed up to one third of anthropogenic CO₂ emissions in the last century (Sabine et al. 2004, Khatiwala et al. 2009) resulting in the reduction of surface ocean pH and carbonate ion concentration ([CO₃²⁻]), an essential building-block for all calcifying organisms. The decline in [CO₃²⁻] is predicted to cause the reduction in calcification across a diverse range of marine calcifiers (Fabry et al. 2008), including planktonic foraminifera (PF) (Bijma et al. 1999, Bijma et al. 2002, Russell et al. 2004, Moy et al. 2009, Manno et al. 2012, Marshall et al. 2013). It is essential to understand the extent to which PF calcification will be affected by this [CO₃²⁻] reduction because they represent up to ~80% of global CaCO₃ in the ocean sediments (Schiebel, 2002) and are vital components of the calcite and organic carbon cycling to the deep ocean. Previously, measurements of shell weights normalised to size (size normalised weights), have been used to estimate shell wall thickness, and therefore calcification rates in planktonic foraminifera. Consequently, increases in size-normalised shell weight have been used to represent faster calcifying shells in higher [CO₃²⁻] environments and hence quantify past changes pCO₂ (Barker and Elderfield, 2002, Gonzalez-Mora et al. 2008, Moy et al. 2009, Naik et al. 2010). However, more recent studies provide conflicting evidence on the environmental variables controlling PF calcification; whilst the influence of temperature may be important in some species (Gonzalez-Mora et al. 2008, Manno et al. 2012), light-availability (Lombard et al. 2010), and nutrient concentrations (Aldridge et al. 2012) have also been proposed as calcification controls in others, leading to significant inter-species variation (Beer et al. 2010a). Increased calcification under conditions optimum to growth may explain some of the variability between species (de Villiers, 2004, Aldridge et al. 2012) so it is essential to understand how changing growth conditions (Lombard et al. 2009, Lombard et al. 2011) (associated with changes in shell size and flux-de Villiers,
Chapter 3: Shell parameters in modern planktonic foraminifera

In this study, we address these issues by individually measuring shell parameters (weight, length and area) in order to effectively size normalise weight to represent changes in shell thickness (shell weight/shell area) and therefore calcification. We investigate shell thickness and size of two planktonic foraminifera species from a biweekly sediment trap time series combined with upper-ocean hydrographic and biogeochemical data collected from a nearby location at Bermuda Atlantic Time Series (BATS) to determine the dominant environmental control(s) on PF shell calcification. Finally, by comparing shell measurement data to species flux data (Salmon et al. 2015) obtained on same samples, we will achieve a unique perspective on the effect of growth-related processes on PF calcification.

3.3. Materials and Methods

3.3.1 The OFP sediment trap time series

We use bi-weekly resolved samples from the 1500m sediment trap collected by the Ocean Flux Program (OFP), located at 31°50'N, 64°10'W in the oligotrophic Sargasso Sea. The OFP traps are just north of the Bermuda Atlantic Time Series (BATS) hydrographic station (31°40'N, 64°10'W) which provides monthly physical and chemical data (http://bats.bios.edu). We analysed two species of symbiont-bearing planktonic foraminifera (33 samples for *Globigerinoides ruber* (pink) and 41 samples for *Orbulina universa*) in the 125-500 µm and 500-1000 µm size fractions collected during two time periods: 1998-2000 and 2008-2010. The seasonal shell parameters of non-symbiont bearing *Globorotalia truncatulinoides* were also measured, but because this species adds
secondary calcite whilst growing and descending into deeper water (Lohmann, 1995, McKenna and Prell, 2004), it is impossible to isolate shell thickness from size changes and hence understand calcification controls on this species ( Appendix 2, Figure 1a-b). Therefore, we cannot discuss controls on the calcification of G. truncatulinoides, but we do use this species' shell measurements to understand trace element incorporation in Chapter 4. The two species analysed in this chapter have comparable ecologies; G. ruber (p) lives in the top ~25m and O. universa lives ~50-100m (Anand et al. 2003, Hemleben et al. 1989) and both have similar growth temperature ranges 20-29 °C (Lombard et al. 2009). Two equivalent 2.5-year intervals (1998-2000 and 2008-2010) were selected to assess seasonal variability in shell thickness and hence calcification in these two depth-stratified species.

3.3.2. Calcification and ‘optimum growth’ proxy

We weighed individual shells in aluminium boats using a Satorius Microbalance (precision = 0.001 mg) before normalising these weights to their respective areas to obtain shell area density (µg/µm²): Number of shells measured per sample were for G. ruber (p) (125-500 µm), average (n = 19), and O. universa (500-1000 µm), average (n = 14). We use ‘ImageJ’ image analysis software to measure the longest distance between two points on the shell (shell size), and silhouette area of shells in the same umbilical orientation. Measured size/area and weight is more effective at normalising shell weights to size than using traditional sieve-size ‘windows’ (Beer et al. 2010b, Marshall et al. 2013) which means actual measured sizes are always larger than sieve sizes (Figure 3.1). The lack of correlation between shell size and area density (Figure 3.2a), shows that area density is an accurate reflection of shell wall thickness in O. universa (calculated using the method of Billups and Spero, (1995)) (Figure 3.2b). We equate larger area densities to thicker, faster calcifying shells (Marshall et al. 2013, Weinkauf et al. 2013) and we use shell size, together with species flux of G. ruber (p) and O. universa (Salmon et al. 2015) in relation to ‘optimum growth conditions’ (Schmidt et al. 2004a, de Villiers, 2004) because we cannot measure growth rate directly.
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Figure 3.1. Sieve size calibration with measured shell size which is always larger than sieve size in the case of irregularly shaped tests like a) G. ruber (pink) and nearest the upper sieve size boundary in rounder tests such as b) O. universa. The error bars represent σ and the minimum and maximum shell size are defined by the Xs.
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Figure 3.2. a) Plot showing effective size-normalisation of area density for both G. ruber (pink) and O. universa. Note the filled circles represent an anomalous year for shell growth through interaction with a cold, cyclonic eddy. b) Validation of area density as a shell thickness proxy using O. universa area density against calculated shell thicknesses from the method by Billups and Spero (1995) assuming a constant 7% porosity for each individual for the Sargasso morphotype (Morard et al. 2009)
There are two genotypes of *O. universa* present at this site, Type I, the Caribbean type which is morphologically characterised by a thicker shell and higher density of more evenly sized pores (~25%) and the Sargasso type characterised by much thinner, transparent shells and a lower pore density with unevenly sized pores (Figure 3.3) (Morard et al. 2009, de Vargas et al. 1999). As these two genotypes are morphologically distinct, we were able to select the more abundant Sargasso genotype using their wall textures, thicknesses, and porosity under high magnification.

**Figure 3.3** High magnification light microscope images taken of the Caribbean (left) and Sargasso (right) *O. universa* genotypes from the OFP sediment trap samples from December and April 2009 respectively.

### 3.3.3 Calcification temperature and depth calculations

Where enough sample material was available, between 130-330 μg (3-8 individuals) of *O. universa* and ~90-220 μg (6-12 individuals) of *G. ruber* (p) were analysed for their oxygen isotope composition to determine calcification temperatures and depth habitats. Analyses were performed on the Finnigan GasBench and Delta+ Advantage stable isotope mass spectrometer (long term standard reproducibility is +/- 0.084‰ for δ¹⁸O and +/- 0.061‰ for δ¹³C) and are reported relative to Vienna Pee Dee Belemnite (V-PDB). Temperature and salinity data from different depth habitats were taken from the Bermuda Atlantic Time Series (BATS) to calculate the δ¹⁸O of calcite in equilibrium with seawater (δ¹⁸Oₛₘ). The
\[ \delta^{18}O_{sw} \] was calculated using the \( \delta^{18}O_{sw} \)-salinity relationship compiled from Atlantic core tops (Arbuszewski et al. 2010, Schmidt et al. 1999) (Equation 3.1):

\[
(3.1) \quad \delta^{18}O_{sw} = -7.69 + 0.238 \times \text{salinity}
\]

Depth habitats were then calculated using equilibrium \( \delta^{18}O \) of calcite and \( \delta^{18}O_{sw} \) and matched to temperature measurements from the BATS database. For \textit{G. ruber} (p), we use the rearrangement of the palaeotemperature equation of O'Neil et al. (1969) and Shackleton, (1974) (Equation 3.2). For \textit{O. universa}, we used the low-light palaeotemperature equation of Bemis et al. (1998), (Equation 3.3):

For \textit{G. ruber} (p):

\[
(3.2) \quad T^{\circ}C = 16.9 - 4.38 (\delta^{18}O_c - \delta^{18}O_{sw}) + 0.1(\delta^{18}O_c - \delta^{18}O_{sw})^2
\]

For \textit{O. universa} (low light):

\[
(3.3) \quad T^{\circ}C = 16.5 - 4.80 (\delta^{18}O_c - \delta^{18}O_{sw})
\]

The \( \delta^{18}O_{sw} \) values were converted from SMOW to PDB by subtracting 0.27 \%o (Hut, 1987). Calcification temperatures derived from \( \delta^{18}O \) were then individually matched to the nearest measured hydrographic temperature (and associated data such as salinity, carbonate chemistry and nutrient concentrations) collected at BATS approximately 2-3 weeks prior to the particular sediment trap sample 'mid-date' (one week after trap opening). A 2-3 week time lag between the hydrographic data and sediment-trap calcification accounts for the \~{}2 week lifespan of planktonic foraminifera (Spero, 1998, Erez et al. 1991) and settling time to the 1500m trap \~{}7 days for \textit{G. ruber} (p) from the surface 25m Takahashi and Bé, 1984) (Appendix 2, Table 1). We find no effect of \([\text{CO}_3^{2-}]\) on \( \delta^{18}O_c \) which has been previously observed in culture studies for \textit{O. universa} (Spero et al. 1997) so no correction was applied here (Figure 3.4). Depth habitats calculated from this study agree well with previous estimates of depth habitats for both of these species
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(Appendix 2, Table 1) with G. ruber (p) ranging from 0-50m, (average 25m) and O. universa ranging from surface waters to 140m, (average ~70m) (Hemleben et al. 1989, Anand et al. 2003).

Figure 3.4. Carbonate ion concentration versus the offset from predicted δ¹⁸O calcite for O. universa (blue) and G. ruber (pink). The δ¹⁸O offset (Δ δ¹⁸O) is defined as δ¹⁸O calcite - δ¹⁸O sw. There appears to be no relationship between Δ δ¹⁸O and [CO₃²⁻] as both O. universa and G. ruber (p) shells are more enriched than expected from calculation using the Spero et al. (1997) calibration for low-light O. universa (shown as the green line, square symbols) where δ¹⁸O = 1.31 - 0.002 × [CO₃²⁻].

3.3.4 Carbonate system parameter calculations

The complete carbonate chemistry system can be computed from a combination of two carbonate parameters (DIC, TA, pCO₂ and/or pH), temperature and salinity. In this case, DIC, TA, temperature, salinity and nutrient concentrations such as P and Si (μmol/kg) from the species depth habitats were inputted into CO₂Sys_v2.1.xls (Pelletier et al. 2007). We applied the carbonic acid dissociation constant of Mehrbach et al. (1973), refit by Dickson and Millero, (1987) and the dissociation constant for HSO₄⁻ (Dickson, 1990). As mentioned in the previous section, we used hydrographic data ~ 3 weeks before the sediment trap sample start; for O. universa average = 27.5 days, G. ruber, pink average = 17 days.
3.3.5. Statistical analysis

We used multiple linear regression analyses in R (http://www.r-project.org) to examine the environmental controls (independent variables) on shell thickness (area density) and shell size (dependent variables). All independent variables were selected based on published calcification controls; temperature (Gonzalez-Mora et al. 2008, Manno et al. 2012), $[\text{CO}_2^\text{aq}]$ (Barker and Elderfield, 2002, Bijma et al. 1999, Bijma et al. 2002, Moy et al. 2009, Marshall et al. 2013), optimum growth conditions (represented here by species flux and size) (de Villiers et al. 2004, Schmidt et al. 2006), and nutrient concentrations (we use chlorophyll a because nutrient concentrations were negligible in the surface waters) (Beer et al. 2010a, Aldridge et al. 2012). First, we eliminated strongly collinear variables determined from Pearson's correlation coefficients (Appendix 2, figures 2-3) (Zuur et al. 2009) before creating a linear model, using the 'lm' function in R (linear model). This linear model was then regressed in a backwards, stepwise fashion using the 'step' function in R, to remove non-contributing variables according to the model's Akaike Information Criterion (AIC) value which determines the simplest and best fitting model when AIC is closest to zero (Akaiiki, 1974).

When two collinear independent variables cannot be eliminated from the model, e.g. temperature and carbonate chemistry, we determined if the residuals from a linear regression of both independent variables significantly improved the $r^2$ or slope values when explaining the variability in the dependent variable. If there is a significant improvement, it shows that a proportion of the variance in the model can be better explained by both temperature and the portion of carbonate chemistry which is not explained by temperature (i.e. carbonate chemistry once the collinearity with temperature has been accounted for). A variation on this technique has been used previously in an attempt to delineate the effects of carbonate chemistry and temperature on the calcification of surface-dwelling PF species in the Cariaco Basin (Marshall et al. 2013).
3.4. Results

3.4.1 Seasonal changes

Figures 3.5-3.6a-d describe the seasonal changes in shell parameters of G. ruber (p) and O. universa in context with their respective fluxes and physical and chemical changes in the water column. In general, G. ruber (p) shells become thicker (higher area densities) (Figure 3.5a) before becoming smaller (Figure 3.5b,c,d) when the SSTs are highest during August-October, before the annual maximum in flux (Figure 3.5d) and [CO$_3^{2-}$]. Smaller G. ruber (p) shell sizes are well correlated with the dip in chlorophyll a concentrations during August (Figure 3.5b), and particularly lower water density at this time (Figure 3.5c). In contrast to G. ruber (p), O. universa shells appear thinner (lower area densities) (Figure 3.6a) and larger (Figure 3.6b,c) during the winter-spring months when temperatures and surface [CO$_3^{2-}$] are lowest. Shell thicknesses then increase, tracking rising temperature/[CO$_3^{2-}$] whilst the trend to smaller shell size tracks the decrease in water density. In contrast to O. universa flux, which reaches a maximum in May, shells become steadily thicker and smaller towards October-November (Figure 3.6d), reaching the smallest and thickest shells when temperature is warmest with the secondary subsurface chlorophyll a peak (Figure 3.6b).
Figure 3.5. Seasonal changes in the area density (light red line), shell size (dark red line) *G. ruber* (pink) and shell flux (dotted, orange line). 

**a)** Area density with respect to $[\text{CO}_3^{2-}]$ and temperature 0-25m. 

**b)** Shell length with respect to temperature 0-25m and chlorophyll a 50-100m representing the depth of the subsurface chlorophyll maximum. 

**c)** Shell length with respect to sigma-theta representing density changes at the subsurface chlorophyll maximum 50-100m. 

**d)** Area density with respect to 'optimum growth' indicators such as shell size and flux. A Stineman function was fitted to all shell measurements for a local smoothing effect (current measurement and 10% of data range). All seasonal changes were averaged from 1998-2000 and 2008-2010.
Figure 3.6. Seasonal changes in the area density (light red line), shell size (dark red line) O. universa and shell flux (dotted, orange line). a) Area density with respect to $[\text{CO}_3^2]\text{^2}$ and temperature 50-100m. b) Shell length with respect to temperature and chlorophyll a 50-100m representing the depth of the subsurface chlorophyll maximum, c) Shell length with respect to sigma-theta representing density changes at the subsurface chlorophyll maximum 50-100m. d) Area density with respect to 'optimum growth' indicators such as shell size and flux. A Stineman function was fitted to all shell measurements for a local smoothing effect (current measurement and 10% of data range). All seasonal changes were averaged from 1998-2000 and 2008-2010, excluding anomalous datapoints from the cyclonic eddy in spring 2010.
3.4.2 Interannual changes

Interannual changes in the fluxes and shell parameters of *G. ruber* and *O. universa* with physical and chemical changes in the water column from 1998-2000 and 2008-2010 can be seen in Table 3.1 below. PF shell size and area density appear to remain seasonally consistent throughout the decade for both species. Typically, the variance in the seasonal thickness and size of *O. universa* is not significantly different between years, varying consistently between 14-16% and 4-6% respectively (e.g. for 1998-99, 2008-09) (Table 3.1). The passage of a cyclonic eddy in 2010 caused the variance of *O. universa* area density and size to increase by up to 30% and 12% respectively (Table 3.1), when *O. universa* shells became 22% smaller and 80% thicker. The average variance in the seasonal thickness of *G. ruber* (p) is similar to *O. universa* and can vary between 11-21% on an interannual basis (Table 3.1).
### Table 3.1

Overview of annual averages and variance of the area density and size for *G. ruber* (pink) and *O. universa.*

<table>
<thead>
<tr>
<th></th>
<th>G. ruber (pink)</th>
<th>O. universa</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Annual average AD</td>
<td>1.27</td>
<td>1.08</td>
<td>1.42</td>
<td>1.18</td>
<td>1.24</td>
<td>0.98</td>
<td>0.81</td>
<td>0.99</td>
<td>0.93</td>
<td>1.24</td>
<td>0.99</td>
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<tr>
<td>σ for AD</td>
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<td>0.20</td>
<td>0.22</td>
<td>0.25</td>
<td>0.20</td>
<td>0.15</td>
<td>0.13</td>
<td>0.14</td>
<td>0.13</td>
<td>0.38</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Relative σ (%)</td>
<td>11</td>
<td>18</td>
<td>15</td>
<td>21</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>30</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Minimum AD</td>
<td>1.11</td>
<td>0.71</td>
<td>1.05</td>
<td>0.91</td>
<td>0.95</td>
<td>0.80</td>
<td>0.58</td>
<td>0.85</td>
<td>0.77</td>
<td>0.88</td>
<td>0.78</td>
<td></td>
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<tr>
<td>Maximum AD</td>
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<td>1.34</td>
<td>1.78</td>
<td>1.60</td>
<td>1.57</td>
<td>1.26</td>
<td>0.95</td>
<td>1.21</td>
<td>1.20</td>
<td>1.75</td>
<td>1.27</td>
<td></td>
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<tr>
<td>Annual average SS</td>
<td>373</td>
<td>363</td>
<td>394</td>
<td>353</td>
<td>371</td>
<td>719</td>
<td>718</td>
<td>718</td>
<td>688</td>
<td>643</td>
<td>697</td>
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<tr>
<td>σ for SS</td>
<td>19</td>
<td>31</td>
<td>36</td>
<td>33</td>
<td>30</td>
<td>43</td>
<td>29</td>
<td>45</td>
<td>42</td>
<td>80</td>
<td>48</td>
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<tr>
<td>Relative σ (%)</td>
<td>5</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>7</td>
<td></td>
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<tr>
<td>Minimum SS</td>
<td>339</td>
<td>302</td>
<td>332</td>
<td>311</td>
<td>321</td>
<td>661</td>
<td>672</td>
<td>668</td>
<td>601</td>
<td>566</td>
<td>634</td>
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<tr>
<td>Maximum SS</td>
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<td>396</td>
<td>435</td>
<td>402</td>
<td>406</td>
<td>773</td>
<td>750</td>
<td>792</td>
<td>736</td>
<td>723</td>
<td>755</td>
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</tr>
</tbody>
</table>

*AD = Area density, all units of which are expressed in 1 x 10^4 μg/μm², including σ. SS = shell size (μm) (longest distance between two points on the shell). The year 2010 was not included for *G. ruber* (pink) because of the limited number of measurements for this species. Slight discrepancies in relative σ may occur from rounding.*
3.4.3 Environmental controls on PF shell parameters

3.4.3.1 Shell thickness/calcification (area density)

By applying multiple regression analysis, we find that carbonate chemistry is most significant in explaining the variability in area density of *G. ruber (p)* and *O. universa* in this study (Table 3.2). However, if we add the residuals of the [CO$_3^{2-}$]-temperature relationship (essentially the portion of [CO$_3^{2-}$] not influenced by its covariance with temperature), the $r^2$ of the model improves from 0.53 to 0.61 suggesting both [CO$_3^{2-}$] and temperature are important in explaining variability in area density. Furthermore, both temperature and [CO$_3^{2-}$] have similar AIC values (-472.85 and -470.01 respectively) and separate regressions of area density with temperature ($r^2 = 0.48, p = << 0.01$) and [CO$_3^{2-}$] ($r^2 = 0.54, p = << 0.01$) support the importance of both temperature and [CO$_3^{2-}$] in explaining the variance in the area density data (Figure 3.7a-b). Interestingly, when both *G. ruber (p)* and *O. universa* from this study are combined with *G. ruber (p)* from Marshall et al. 2013, we find that temperature explains most of the variation in area density ($r^2 = 0.63, \text{AIC} = -783, p << 0.01$) compared to [CO$_3^{2-}$] ($r^2 = 0.47, \text{AIC} = -795, p << 0.01$) and the combination of both temperature and [CO$_3^{2-}$] explains slightly more of the variance in area density than temperature alone ($r^2 = 0.66, p << 0.01$).
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### Area density

<table>
<thead>
<tr>
<th>Variable</th>
<th>G. ruber (p), O. universa</th>
<th>O. universa</th>
<th>G. ruber (p), O. universa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This study</td>
<td>This study</td>
<td>This study, Marshall et al. (2013)</td>
</tr>
<tr>
<td>[CO₃²⁻]</td>
<td>0.00901 *</td>
<td>0.00958 *</td>
<td>3.50 x 10⁻³</td>
</tr>
<tr>
<td>Calcification</td>
<td>0.0207</td>
<td>0.0268</td>
<td>0.0395 ***</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shell size</td>
<td>1.64 x 10⁻⁴</td>
<td>-8.90 x 10⁻⁴</td>
<td>-</td>
</tr>
<tr>
<td>Species flux</td>
<td>-3.43 x 10⁻³</td>
<td>-0.0235</td>
<td>-</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>-3.14 x 10⁻⁵</td>
<td>-2.83 x 10⁻³</td>
<td>-</td>
</tr>
<tr>
<td>Multiple r²</td>
<td>0.62</td>
<td>0.51</td>
<td>0.68</td>
</tr>
<tr>
<td>Adjusted r²</td>
<td>0.58</td>
<td>0.42</td>
<td>0.66</td>
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</tbody>
</table>

Table 3.2. Multiple linear regression results displaying slope coefficients (sensitivities) of area density with independent variables for G. ruber (p) and O. universa from this study and also with G. ruber (p) from Marshall et al. (2013). There was no shell size, flux or chlorophyll a data to compare with Marshall et al. (2013). Adjusted r² accounts for sample size and number of independent variables. Variables shown in bold significantly contribute to the model * p < 0.05, ** p < 0.001, *** p = 0

#### 3.4.3.2 Shell size

Multiple linear regression results show that sigma-theta (water density) (AIC = 213.48) and temperature (AIC = 209.44) are the dominant controls on variability in shell size (Table 3.3), although species flux may also contribute (AIC = 188.38). Separate regressions with temperature and sigma-theta suggest that sigma-theta explains more of the variance than temperature (r² = 0.75) (Figure 3.7c-d). No single parameter explains the majority of the variance in the shell size of O. universa only (Table 3.3).
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Figure 3.7. Relationships between shell parameters and environmental parameters taken from respective calcification depths deduced from δ¹⁸O_{calcite}. For area density a) Hydrographic temperature, b) [CO₃²⁻], and shell size c) hydrographic temperature, d) Sigma-theta (water density). Species G. ruber (pink) and O. universa are colour-coded pink and blue respectively. Open circles are not included in the regression; blue, open circles represent O. universa during spring 2010 when a cyclonic eddy passed over the sediment trap coinciding with anomalously thick shells. Pink, open circles represent a G. ruber (pink) anomaly during summer 2008. Error on measurements were calculated using AD/Shell length x (1/n).
### Shell size

<table>
<thead>
<tr>
<th>Variable</th>
<th>G. ruber (p)</th>
<th>O. universa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This study</td>
<td>This study</td>
</tr>
<tr>
<td>Sigma-theta</td>
<td>134.43 ***</td>
<td>18.7</td>
</tr>
<tr>
<td>Calcification</td>
<td>-32.32 ***</td>
<td>-5.74</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species flux</td>
<td>9.93</td>
<td>75.6</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>0.28</td>
<td>1.86 x 10^-3</td>
</tr>
<tr>
<td>Multiple r^2</td>
<td>0.78</td>
<td>0.09</td>
</tr>
<tr>
<td>Adjusted r^2</td>
<td>0.75</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

Table 3.3 Multiple linear regression results displaying slope coefficients (sensitivities) of shell size with independent variables for G. ruber (p) (n = 6) and O. universa (n = 19) from this study. The left column describes variations in both species and the right column, just O. universa. Adjusted r^2 accounts for sample size and number of independent variables. Variables shown in bold significantly contribute to the model * p < 0.05, ** p < 0.001, *** p = 0

### 3.5 Discussion

#### 3.5.1 Controls on PF shell calcification

##### 3.5.1.1 Temperature and carbonate chemistry

Results of multiple linear regression show that incorporating both temperature and [CO_3^{2-}] into the model improves the fit by ~15% suggesting the effect of both variables are important in the calcification of both O. universa and G. ruber (p) (Figure 3.7a-b) which is consistent with previous studies on cultured Neogloboquadrina pachyderma (Manno et al. 2012) and over glacial-interglacial timescales on Globigerina bulloides and Globigerinoides ruber (w) (Gonzalez-Mora et al. 2008). Despite this, most culture studies done on O. universa (Caribbean genotype) conclude that changes in carbonate chemistry primarily control shell calcification inferred from shell thickness (Bijma et al. 1999, Bijma et al. 2002, Russell et al. 2004, Lombard et al. 2010). However, according to these culture
experiments, we would only expect to see 0.9µm change in shell thickness of *O. universa* given our typical seasonal range in [CO$_3^{2-}$], but we actually see 2.33µm seasonal change in shell thickness (calculated using the method of Billups and Spero, 1995). The 1.43µm discrepancy between the expected and observed seasonal change in shell thickness of *O. universa* could be caused by temperature (with minor contributions from other variables discussed later).

To test the temperature effect on calcification, we analysed changes in shell thickness from cultured *O. universa* (Russell et al. 2004) over the experimental temperature range (15-25°C) in context with seasonal temperature changes experienced in this study (Figure 3.8). Regardless of changes in [CO$_3^{2-}$], we show that the shell thickness of *O. universa* in this study and in culture, has a tendency to increase at higher temperatures, comparable to the growth curves (~18-22°C) modelled by Lombard et al. (2009) (Figure 3.8).

However, if temperature and carbonate chemistry were the only controls on the calcification of *G. ruber* (p) and *O. universa*, we would expect the variance in *G. ruber* (p) area density to be smaller than *O. universa* because *G. ruber* (p) only experiences 26-28°C and a small range in [CO$_3^{2-}$] of ~15 µmol/kg (Figure 3.9a,b), compared to the 18-25°C and ~30 µmol/kg experienced by *O. universa* (Figure 3.10a,b). Surprisingly the range in *G. ruber* (p) area densities is similar, if not larger than in *O. universa* (Table 3.1). Clearly, there are other controls on shell thickness that have not been described in the model, which we will explore in the next section.
Chapter 3: Shell parameters in modern planktonic foraminifera

Figure 3.8. The effects of temperature on the calculated thickness of *O. universa* (see caption figure 3.2 for details) from this open-ocean study (red- Sargasso genotype) and measured thickness from cultured tests in Russell et al. (2004) (black- Caribbean genotype). Mean values for each temperature treatment from the culture work are represented by the large, black, filled circles. The spread in values around the mean for the culture work represent a range in different [CO$_3^{2-}$] treatments, shown on the figure in µmol/kg. From this study, the large, red filled circles represent mean temperatures within 1°C groupings and associated wall thicknesses. The average [CO$_3^{2-}$] range for this study is shown in red on the figure (213-241 µmol/kg) and the range in temperature varied between 19-25°C.

3.5.1.2 Optimum growth conditions

If faster calcification inferred from thicker shells were synchronous with 'optimum growth conditions', we would expect to see larger fluxes of bigger, thicker shells. However, our MLR results show no significant relationship between changes in size and/or flux and area density of either *G. ruber* (p) or *O. universa* (Table 3.2) and fluxes of both species occur over a range of area densities, temperatures and [CO$_3^{2-}$] (Figures 3.9-3.10a-b) arguing against greater calcification during 'optimum growth' conditions (de Villiers, 2004). Our results agree with Weinkauf et al. (2013) who also showed that the area densities of *O. universa* remained unchanged during environmental stress.
Figure 3.9. Histograms for *G. ruber* (p) showing distribution of area density (filled, black circles) and *G. ruber* (p) flux (Xs) with a) calcification temperatures and b) \([\text{CO}_3^{2-}]\) (grey bars). c) Distribution of shell size (filled, black circles) with calcification temperatures (grey bars). An outlier (shown as a filled, pink circle) coincides with an unseasonal high shell flux. Error on measurements were calculated using area density or shell size x (1/n).
Figure 3.10. Histograms for *O. universa* showing distribution of area densities (filled, black circles) and *O. universa* flux (Xs) with a) calcification temperatures and b) \([\text{CO}_3^{2-}]\) (grey bars). c) Distribution of shell size (filled, black circles) with calcification temperatures (grey bars). Blue points represent shell measurements coinciding with a cyclonic eddy. Error on measurements were calculated using area density or shell length \(x (1/n)\).
Despite the lack of correlation between ‘optimum growth’ and calcification, some of our results suggest that changes in foraminiferal growth processes, inferred from shell size and flux, are related to shell calcification, inferred from area densities. For instance, we show that shell size in G. ruber (p) and O. universa varies primarily with water density and/or temperature with shells becoming thicker and smaller in warmer, less dense waters (Figures 3.5-3.6, Table 3.3). Water density may determine the calcification depth of shells, and thus their access to food phytoplankton (indicated here using chlorophyll a concentrations) or in the case of symbiont-bearing species such as G. ruber (p) and O. universa, access to light. However, in this were the case, we would expect a significant relationship between shell size and chlorophyll a concentrations (Table 3.3). Equally, the presence of smaller shells in warmer waters (Figure 3.5c, 3.6c 3.7c) is contrary to the paradigm view that increased temperatures should yield larger shells (Schmidt et al. 2004a, 2004b, 2006) suggesting that shell size changes are indicative of a more complex process. From our observations, G. ruber (p) shells become thicker before becoming smaller in warmer, less dense waters (Figure 3.5b-c), suggesting that shell size is reduced in order to counteract the greater settling velocity caused by thicker shells which sink faster in warmer waters, consistent with a recent study (Caromel et al. 2014).

Salmon et al. (2015) showed that upwelled nutrients and turbulence caused by transient cyclonic eddies in the Sargasso Sea can spark exceptional growth periods in certain species of planktonic foraminifera as well as phytoplankton (Wiebe and Joyce, 1992, Olaizola et al., 1993, McNeil et al., 1999, Letelier et al., 2000, Seki et al., 2001, Sweeny et al., 2003). During spring 2010, a cyclonic eddy triggered greater fluxes of O. universa which grew smaller and thicker potentially to regulate their buoyancy in a more turbulent water column (Figure 3.7), consistent with observations in upwelling areas where PF grow smaller shells to adapt their buoyancy and regulate exposure to light (Bé et al. 1973, Bijma et al. 1992, Ortiz et al. 1995). Alternatively, higher fluxes of thicker-shelled O. universa during the eddy (probably due to the prolonged subsurface chlorophyll a
Chapter 3: Shell parameters in modern planktonic foraminifera

maximum), could be indicative of faster growth and therefore calcification rates. Equally, low surface water temperatures during the eddy (18.3-19.3°C) were outside the optimum growth temperature range for *O. universa* (20-29°C, Lombard et al. 2009), which could have caused a reduction in shell size. Interestingly, when *O. universa* is growing below its optimum growth temperature range, calcification appears to become more sensitive to carbonate chemistry changes (Figure 3.7b). Increased calcification sensitivity outside of the optimum 'thermal window' has previously been observed in cultured *N. pachyderma* (Manno et al. 2012), and suggests a non-linear relationship between calcification and environmental parameters.

Finally, we acknowledge that measuring the size and flux may not be wholly representative of the complex process of foraminiferal growth (Aldridge et al. 2012, Lombard et al. 2011) and suggest further research is needed to define the interactions between calcification and shell growth.

3.5.1.3 Causes of other variation

So far, we have described environmental variables which best explain variance in shell sizes and thickness of both species. Whilst shell size is generally well described by temperature and water density ($r^2 = 0.75$), a significant portion of the variance in shell thickness (39% both species, 57% *O. universa*) is not explained by factors inputted into the model. Salinity is not expected to significantly contribute to the remaining variation in shell calcification because its seasonal range is limited at this site (~0.6 PSU at 0-25m) and it co-varies with at least one of the included independent variables, so it is already essentially represented in the model. Previous studies have shown light intensity can govern calcification in symbiotic species (Spero, 1988, Bijma et al. 1999, 2002, Russell et al. 2004, Lombard et al. 2010), but we find no significant relationship between light intensity and area densities of either *G. ruber* (p) or *O. universa* ($r^2 = 0.30$) compared with area densities and corresponding $[CO_3^{2-}]$ from the same time period ($r^2 = 0.78$), (data only available for 1999-2000). We attribute most of the unexplained variability in shell...
thickenss to the interplay of non-linear relationships probably caused by overprinting of various environmental and biological controls on shell calcification.

### 3.5.2 Calibration comparison

From Figure 3.7a-b, it is apparent that both *G. ruber* (p) and *O. universa* plot on the same temperature and [CO$_3^{2-}$] regression line which is perhaps surprising, as other studies have observed distinctive species-specific calcification responses to various environmental variables (Beer et al. 2010a, Marshall et al. 2013). Both *G. ruber* (p) and *O. universa* bear symbionts and have similar growth rates (Lombard et al. 2009), which could explain their comparable calcification responses. Figure 3.11a compares the wide range in sensitivities of area density-[CO$_3^{2-}$] calibrations from various culture and sediment-trap studies for both species *O. universa* and *G. ruber* (p). Interestingly, the sensitivities of PF calcification to [CO$_3^{2-}$] from oligotrophic (this study) and upwelling sites (Marshall et al. 2013) are comparable despite different seasonal balances of other environmental variables. By comparison, changes in calcification are ~4 times less sensitive in culture studies (Figure 3.11b) which could be due to 1. Different genotypes of *O. universa*; this study uses the Sargasso genotype (Type II) (de Vargas et al. 1999), whereas culture studies generally use the Caribbean genotype (Type I) (Bijma. J., pers. comm). Both of these *O. universa* genotypes have different wall thicknesses (Figure 3.3) (Morard et al. 2009), weight-length relationships (Marshall et al. 2015) and therefore different area density-[CO$_3^{2-}$] relationships. 2. Also, synergic effects of environmental variables e.g. [CO$_3^{2-}$]/temperature/food phytoplankton/light intensity, in a natural setting could create a greater net change in calcification when compared to limited variables changing in Bijma et al. (1999, 2002) and Russell et al. (2004). The synergic effects of variables on calcification has been observed in a few culture studies which found greater net change in calcification when changing two environmental variables; Manno et al. (2012) described the combined effects of temperatures and [CO$_3^{2-}$] on the calcification of *N. pachyderma*, whilst Lombard et al. (2010) noted the higher calcification rates of *G. sacculifer* and *O. universa* under higher irradiance, despite similar [CO$_3^{2-}$].
Figure 3.11. a) Calibration comparison showing the ranges of area density and $[\text{CO}_3^2]$ for both *G. ruber* (pink) and *O. universa* from this study with the 355-500µm *G. ruber* (p) calibration from Marshall et al. (2013) and calibrations for *O. universa* from culture (Bijma et al. 1999, 2002, Russell et al. 2004). Size-normalised shell weights from Bijma et al. (1999, 2002) were converted to area density using the radius and weight of the shells tests cultured under $<100\mu\text{mol/kg}$ $[\text{CO}_3^2]$ conditions were excluded. b) The offset between culture (Bijma et al. 1999, 2002, Russell et al. 2004- *O. universa*) and sediment-trap calibrations (This study *O. universa*, *G. ruber* (p), Marshall et al. 2013- *G. ruber* (p)).
3.6. Conclusions

We have explored controls on the shell calcification of two depth-stratified, symbiont-bearing foraminifera (G. ruber, pink and O. universa) through discussion of changes in their area density (shell thickness) a calcification proxy, in relation to changes in shell size and flux, the 'optimum growth' indicators, and environmental variables. Our main findings are as follows:

- Using multiple linear regression, we find that $[\text{CO}_3^{2-}]$ and temperature are the dominant environmental controls on the calcification of both species at this study site.

- There is no relationship between shell flux and shell thickness disproving an 'optimum growth' control on calcification, but we do observe smaller, thicker shells in warmer, lower density water consistent with planktonic foraminifera regulating their size to alter buoyancy in the water column.

- The relationship between G. ruber (p) and O. universa shell thickness with $[\text{CO}_3^{2-}]$ from sediment-trap studies appears ~4 times more sensitive than equivalent culture relationships. We suggest that this offset is caused by either O. universa genotypic differences between culture and open-ocean studies in, or the synergic effects of different variables, especially temperature and $[\text{CO}_3^{2-}]$, co-varying in natural settings and creating larger changes in area density/shell thickness and hence calcification.

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Cushman Foundation for their financial support through the Johanna Resig Foraminifera
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Bermuda Atlantic Time Series (most recently by grant OCE-0801991).

Contributions

K. S. selected the sampling periods, collected the data and interpreted results. P.J. aided
K.S. in analysing the initial results, P. A. conceived the project idea, and P.A. and P.F.S.
secured funding. M. C. provided the sediment trap samples. K.S., P.A., & P.F.S.
contributed their relevant expertise to constructing the manuscript.
"Those who contemplate the beauty of the earth find reserves of strength that will endure as long as life lasts."
4. Testing the reliability of trace element proxies in planktonic foraminifera

This Chapter has been written to submit to *Earth and Planetary Science Letters*:

4.1 Abstract

We have examined the controls on several planktonic foraminifer-hosted trace element proxies using sediment trap samples from the Sargasso Sea in conjunction with measured hydrographic data from the same area. We also tested the control of shell flux, size, and thickness of three depth-stratified species (*G. ruber* (p), *O. universa*, *G. truncatulinoides*-encrusted and non-encrusted) on trace element incorporation from the same samples. Unlike another study from this site, we find a significant temperature control on Mg/Ca in *O. universa*, although it is positively offset from other species by ~3 mmol/mol. Sr/Ca and Li/Ca are not reliable temperature proxies in planktonic foraminifera; Although Sr/Ca responds to temperature in all species, it is 5-6 times less sensitive to temperature than Mg/Ca, and counter-intuitively, Globorotaliid species are more enriched than surface-dwelling species. Likewise, Li/Ca in *G. ruber* (p) and non-encrusted *G. truncatulinoides* is 3 times less sensitive to temperature than Mg/Ca, with no sensitivity in *O. universa*. Li/Ca has no relationship with [CO$_3^{2-}$], shell thickness or size, discounting a calcification rate control on incorporation of Li. The proxies of ocean carbonate equilibria, B/Ca and U/Ca show little or no correlation with carbonate system parameters between different studies and within this study. We find a strong positive relationship between shell thickness and B/Ca in all species suggesting a calcification rate control on boron partitioning, supported by previous observations of less efficient trace element discrimination at higher calcification rates. The observed secondary temperature control on B/Ca could also be explained through faster calcifying shells at higher temperatures. We also observe a
weak shell size dependency of B/Ca in *G. ruber* (p) only, probably because larger shells also tend to calcify quicker in this particular species. U/Ca displays strongly species-specific, shell size dependent relationships in all species but no relationship with shell thickness, suggesting a biological, rather than calcification rate control on its incorporation. Our results advocate for caution when using B/Ca and U/Ca as proxies for the ocean carbonate system, and we recommend limiting the thickness as well as the size ranges of planktonic foraminifera shells in order to determine the dominant environmental controls on trace element incorporation into their shells.
4.2 Introduction

The trace element composition of planktonic foraminifera records a wealth of information about the marine environment in which it grew. Consequently, trace element signatures have been used extensively in palaeoceanography to reconstruct past seawater temperature (Nürnberg et al. 1996, Elderfield and Ganssen, 2000, Barker et al. 2005, Yu et al. 2008, Lear et al. 2010) carbonate chemistry (Yu et al. 2007, 2013, Foster, 2008, Tripati et al. 2009), salinity (Hall and Chan, 2004a, Bahr et al. 2013) and nutrient concentrations (Elderfield and Rickaby, 2000, Yu et al. 2013). However, despite the wider application of these geochemical proxies, discrepancies still remain within and between different calibration studies, because of different sample types (e.g. Henehan et al. 2015), study sites (e.g. Ni et al. 2010), observational timescales (e.g. Elderfield et al. 2000), species-specific relationships (e.g. Yu et al. 2007), sample size fractions (Elderfield et al. 2002, Friedrich et al. 2012, Babila et al. 2014), and especially between open-ocean and culture studies (Allen et al. 2012 review). We have compiled the full extent of all reported controls on different trace element proxies in Table 4.1.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>Culture + Core tops</td>
<td><em>N. Pachyderma</em></td>
</tr>
<tr>
<td>Culture Core tops</td>
<td><em>G. bulloides</em></td>
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<tr>
<td>Culture</td>
<td><em>O. universa</em></td>
</tr>
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<td><em>O. universa</em></td>
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<td><em>G. bulloides</em></td>
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<tr>
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<tr>
<td>Calcification/Growth Rate Plankton</td>
<td>planktonic</td>
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<tr>
<td>Calcification/Growth Rate</td>
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<tr>
<td>[CO$_3^{2-}$] (below 200 µmol/kg) Culture</td>
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</tr>
<tr>
<td>[CO$_3^{2-}$] (below 200 µmol/kg)</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td><em>G. truncatulinoide</em></td>
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<td>Calcification temperature Sediment</td>
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## Chapter 4: Trace element proxies in planktonic foraminifera

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<td>Henehan et al. 2015</td>
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<td>Down core</td>
<td>Various benthic species, N. pachyderma</td>
<td>Yu and Elderfield 2007</td>
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<td>Lea and Spero 1994</td>
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<td></td>
<td>Culture</td>
<td>G. sacculifer</td>
<td>Lea and Spero 1994</td>
</tr>
<tr>
<td></td>
<td>Core</td>
<td>N. pachyderma</td>
<td>Hall and Chan 2004b</td>
</tr>
<tr>
<td>Depth habitat</td>
<td>Tows, Core tops+sediment trap</td>
<td>Globorotaliids</td>
<td>Lea and Boyle 1991</td>
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</tbody>
</table>

<table>
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<tr>
<th>U/Ca Seawater U/Ca Calcification temperature</th>
<th>Culture</th>
<th>G. calida</th>
<th>Russell et al. 1994</th>
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<td>Russell et al. 1996</td>
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<td>Core tops and down-core</td>
<td>G. bulloides</td>
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<td>Yu et al. 2008</td>
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<td>G. inflata</td>
<td>N. pachyderma</td>
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Table 4.1. A summary of the formative literature about controls on trace element incorporation in mainly planktonic foraminifera.

Table 4.1 shows that there is still more work needed to fully resolve dominants controls on most trace elements and especially B/Ca incorporation in planktonic foraminifera.

Although we will be examining all trace elements in this study, we will focus particularly on the behaviour of B/Ca because it's use as a carbonate system proxy is still hotly debated.

Theoretically, B/Ca exists in seawater as two species boric acid \([\text{B(OH}_3\text{)}]\) and borate ion \([\text{B(OH}_4\text{)}^-]\), the proportions of which are pH dependent:

\[
(4.1) \text{B(OH}_3\text{)} + \text{H}_2\text{O} \rightleftharpoons \text{B(OH}_4\text{)}^- + \text{H}^+
\]

As the \([\text{B(OH}_4\text{)}^-]\) is a charged ion, it is thought this is the only species which substitutes for \([\text{CO}_3\text{}}^2\text{]\), and this has recently been supported (Klochko et al. 2009, Branson et al. 2015, Nir 80
et al. 2015). Increasing pH therefore leads to a greater incorporation of B in CaCO$_3$ lattice:

\[(4.2) \text{CaCO}_3\text{solid} + \text{B(OH)}_4^{-\text{aqueous}} \rightarrow \text{Ca(HBO}_3)_\text{solid} + \text{HCO}_3^{-\text{aqueous}} + \text{H}_2\text{O}\]

Leading to an exchange distribution coefficient ($K_D$) as defined by Zeebe and Wolf-Gladrow, (2001) and Yu et al. (2007):

\[(4.3) K_D = \frac{[\text{B/Ca}]_\text{solid}}{[\text{B(OH)}_4^-/\text{HCO}_3^-]_\text{seawater}}\]

However, there is considerable evidence that argues against a primary carbonate system control on B/Ca ratios including: (i) The lack of correlation between B/Ca and pH-dependent boron isotope composition (Foster, 2008) and carbonate system parameters (Babila et al. 2014), (ii) Higher B/Ca in low pH upwelling regions (Naik and Naidu, 2014), (iii) Species-specific sensitivities of B/Ca to carbonate chemistry in culture compared to open-ocean studies (Yu et al. 2007, Allen et al. 2012 review) (iv) Size-dependent fractionation of B/Ca (Yu et al. 2007, Babila et al. 2014, Henehan et al. 2015).

In order to address these confounding observations, we utilise two 2.5 year intervals of seasonal biweekly sediment trap samples collected by the Oceanic Flux Program (OFP) from a 1500m mooring located in the Sargasso Sea. To obtain a larger environmental gradient over which to test trace element incorporation, we use three depth-stratified species living between 0-400m over all seasonal cycles. We will combine temperature, salinity, and carbonate system data collected from the nearby Bermuda Atlantic Time Series (BATS) with the shell flux, shell parameters (Chapter 3) and trace element geochemistry data with the aim to:

1. Identify the dominant environmental, ecological and seawater chemistry controls on Sr/Ca, Mg/Ca, U/Ca, Cd/Ca, Li/Ca, Ba/Ca and especially B/Ca as a carbonate system proxy planktonic foraminifera by testing variations with shell size, area
density (carbonate system proxy) and flux measurements on the same samples from previous chapters.

2. Quantify relationships between trace elements and dominant controls to derive empirical relationships using measured hydrographic data from BATS.
4.3 Oceanographic Setting

The OFP site is located in the Sargasso Sea within the North Atlantic gyre. Solar insolation drives the large sea surface temperature range at this site (~29°C in July-September to 19°C from January-March), and a small surface salinity range (0.4 PSU) (Figure 4.1a-b). Changes in carbonate chemistry at the surface are small (~225 μmol/kg in March-April and ~248 μmol/kg in September-October) but greater with depth reaching 180-190 μmol/kg at 400-500m (Figure 4.1c) and are driven by physical mixing, gas exchange and biological production (Bates et al. 1996, 1998). The mixed layer is primarily <50m for most of the year with an underlying subsurface chlorophyll maximum at approximately 80-100m depth. During the winter months, the mixed layer erodes to a maximum depth of 250-400m and the site becomes a CO₂ sink (Bates, 2007). During summer, thermal stratification is at a maximum, and the site becomes a CO₂ source to the atmosphere, (Bates et al. 1996, 1998, Bates, 2007).
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Figure 4.1. Annual cycles of a) temperature, b) salinity, c) $\left[\text{CO}_3^2^-\right]$ from BATS hydrographic station in the Sargasso Sea, averaged over the same time period as the sediment trap deployments (1998-2000 and 2008-2010). Numbers on the plots are water depths in metres.
4.4 Materials and Methods

4.4.1 The OFP sediment trap material and Bermuda Atlantic time series

The OFP mooring is located at 31°50'N, 64°10'W, about 55 km southeast of Bermuda and north of the BATS (31°40'N, 64°10'W) (see Figure 2.1, Salmon et al. 2015). We selected two equivalent 2.5 year intervals (1998-2000 and 2008-2010) at 1500m water depth to capture seasonal variations in the shell parameters and geochemical composition of three, depth-stratified species of foraminifera from the 125-500 µm and 500-1000 µm size fractions (G. ruber (pink), O. universa and Globorotalia truncatulinoides, non-encrusted (nc) and encrusted (c)). Before foraminiferal shells were destroyed for geochemical analyses, we first measured shell area densities (proxy for shell thickness and calcification rate), as discussed in Chapter 3, section 3.3.2. Shells used for geochemical analysis were selected from a narrow size fraction; Average ± standard deviation for G. ruber (p) = 400 µm ± 49 µm, O. universa = 748 µm ± 49 µm, G. truncatulinoides (nc) = 509 µm ± 106 µm, G. truncatulinoides (c) = 642 µm ± 112 µm. G. truncatulionides (non-encrusted and encrusted) vary in size from 347-774 µm explaining the larger variance observed in this species. Chapter 3, figure 3.1 and Appendix 2, Figure 1 both provide guidance for conversion of digitally measured shell size to traditional sieve sizes. We used the right-coiling variety of G. truncatulinoides in this study which all belong to the same genetic group (Type II) (Ujiié et al. 2010), as this was the only genotype present at our study site (de Vargas et al. 2001). By selecting species with variable depth habitats (0-400m), a large range in depth-adjusted (see section 4.4.3-4.4.4) hydrographic data is captured to compare with species’ geochemical compositions (http://bats.bios.edu).

4.4.2 Sample preparation and elemental analysis

(See appendix 3 for full method)

Planktonic foraminifera shells were gently crushed between two glass slides, following the method of Russell et al. (2004). Between 3 and 23 shells (depending on weight) were
crushed for analyses to give ~130-1600 μg of material which was split between trace element and stable isotope analyses. Samples were then oxidised to remove organic matter followed by a weak acid leach step prior to final dissolution (see Appendix 3 methods). Trace element analysis (Li, B, Mg, Al, Sr, Cd, Ba, U, Na, Mn) was carried out at the Godwin Laboratory at Cambridge University following the method of Yu et al. (2005). Calcium concentrations were first determined using an Inductively-Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Aliquots of the same solution were then diluted to 10 ppm [Ca] to minimise matrix effects during analysis on the Inductively-Coupled Plasma Mass Spectrometry (ICP-MS), according to Misra et al. (2014). A mixed matrix of 0.1 HNO₃ and 0.3 M HF was used in this study in order to reduce the B/Ca memory effect. Standards were prepared with ultrapure water, and allowed a B/Ca detection range between ~ 9.0-250 μmol/mol. Standard and blank solutions were measured every 5 samples producing a long-term B/Ca internal precision < 1.0% (2σ) and external precision < 4.0% (2σ) (Misra et al. 2014). Other trace element external precisions are shown in Appendix 2, Table 1. Typical B blank levels in this run were < 2% of [B] in foraminifera samples and an in-house standard was used to correct for drift over the run.

4.4.3 Calcification temperature and depth calculations
Calcification temperatures were determined from analysing the δ¹⁸Ocalcite. This method is described fully in Chapter 3, section 3.3.3 (using equation 3.2 for G. truncatulinoides). For samples where no stable isotope data were available, we used Mg/Ca to calculate the calcification temperatures and associated depth habitats with species-specific Mg/Ca-temperature calibration equations from Anand et al. (2003), (Appendix 2, Table 1):

(4.4) G. ruber (p) 350-500μm sieve size:

\[ T (°C) = \ln \left( \frac{\text{Mg/Ca}}{0.73} \right) / 0.067 \]
(4.5) *O. universa* (350-500\( \mu \)m sieve size):

\[
T ({}^{\circ}C) = \ln \left( \frac{\text{Mg/Ca}}{0.595} \right) / 0.090
\]

(4.6) *G. truncatulinoides* (300-500\( \mu \)m sieve size):

\[
T ({}^{\circ}C) = \ln \left( \frac{\text{Mg/Ca}}{0.359} \right) / 0.090
\]

Previous studies have observed species-specific salinity and \([\text{CO}_3^{2-}]\) controls on Mg/Ca (Lea et al. 1999, Russell et al. 2004, Mathien-Blard and Bassinot 2009, Arbuszewski et al. 2010, Hönisch et al. 2013). In this study *G. ruber* (p) experiences salinity changes of 0.43 PSU and \([\text{CO}_3^{2-}]\) of 18 \(\mu\)mol/kg, and *O. universa* experiences changes of 0.28 PSU and 31 \(\mu\)mol/kg over a seasonal cycle. These changes in salinity and \([\text{CO}_3^{2-}]\) are equivalent to a 1.42 ± 0.73\% and 1.23 ± 0.64\% change in Mg/Ca sensitivity in *G. ruber* (p) and *O. universa* respectively (Hönisch et al. 2013) which both fall within the long term relative standard deviation of Mg/Ca measurements (Appendix 2, Table 1) so we would not expect these variables to affect Mg/Ca but will test this in section 4.5.3.

### 4.4.4 Carbonate parameter calculations

The complete carbonate chemistry was calculated using CO2Sys_v2.1.xls (Pelletier et al. 2007) from a combination of two carbonate parameters (dissolved inorganic carbon (DIC), total alkalinity (TA), \(p\text{CO}_2\) and/or pH) and temperature and salinity. We applied the carbonic acid dissociation constant of Mehrbach et al. (1973), refit by Dickson and Millero, (1987) and the dissociation constant for \(\text{HSO}_4^-\) (Dickson, 1990). We used depth-adjusted hydrographic data ~ 3 weeks before the sediment trap sample start, to compensate for the average 2-3 lifecycle of planktonic foraminifera (Erez et al. 1991, Spero, 1998) so that *O. universa* average = -27 days, *G. ruber*, pink average = -20 days and *G. truncatulinoides* average = -23 days (Appendix 2, Table 1).
4.4.5. Statistical analysis

4.4.5.1 Multiple Linear Regression

Multiple linear regressions were used as before (Chapter 3, section 3.3.5) to test which of the independent environmental (e.g. carbonate chemistry, temperature, salinity) and ecological (shell parameters e.g. flux, area density/shell thickness, size- Chapter 3) explain the variations in the dependent variables i.e. the trace elements, (Mg/Ca, B/Ca, Li/Ca, Sr/Ca, U/Ca, Ba/Ca, Cd/Ca). We selected independent variables based on previous observations of temperature (Elderfield and Ganssen, 2000, Anand et al. 2003, Barker et al. 2005, Yu et al. 2008, Cléroux et al. 2008), salinity (Arbuszewski et al. 2010, Hönsisch et al. 2011, Hönsisch et al. 2013), carbonate chemistry, (Yu et al. 2007, 2013; Allen et al. 2011, 2012), crust formation (Spear et al. 2011, Jonkers et al. 2012, Bolton and Marr, 2013) and size-fraction fractionation (Elderfield et al. 2002, Ni et al. 2007, Friedrich et al. 2012) related to calcification/growth rate (we use area density/shell size as a proxy) (Lea et al. 1999, Hall and Chan, 2004) affecting the incorporation of trace element into the foraminiferal test. The more sensitive the dependent variable is to changes in the independent variable, the greater the value of the slope coefficient in the regression (Table 4.2).

4.4.5.2 Principal Component Analysis

Whilst using multiple linear regressions to shed light on extra and intra-test controls on trace element incorporation, we also utilise Principal Component Analysis (PCA) (using the 'prcomp' function in R), to visually display the distributions of trace elements in relation to environmental parameters. The principal component (or PC1) is the axis which explains the most variance, then the second axis (PC2) which is drawn orthogonally to the first, and so on. This occurs in multidimensional space with one dimension for each of the variables included in the analysis (Dytham, 2011- stats book). The loading of the variables is expressed through the length of red arrows relative to the axis score, whilst
the angle of the arrows relative to each other define the relationship between the variables e.g. if two arrows are at 180° to one another, the two variables are inversely correlated, whereas two arrows varying at the same angle show that both variables are proportionally correlated.

4.5 Results

4.5.1. Seasonal changes in stable isotopes

Figure 4.2a-b describes the seasonal changes in oxygen and carbon isotopes of foraminiferal calcite measured over both of the sediment trap deployment periods. The seasonal change in $\delta^{18}O$ varies by up to $\sim$2.0‰, consistent with the calcification depth temperatures of each species (Figure 4.2a), on average 26 m for *G. ruber* (p) and 68 m for *O. universa*. The $\delta^{13}C$ also undergoes a seasonal $\sim$2.0‰ shift from negative to more positive values from spring to summer, mirroring the higher spring to lower summer DIC and chlorophyll *a* concentrations (Figure 4.2b). Interestingly, the $\delta^{18}O$ of non-encrusted *G. truncatulinoides* are generally 0.5-1‰ lighter than their encrusted equivalents, corresponding to calcification depths of 88 m and 411 m respectively, with some non-encrusted individuals calcifying at 0m (Appendix 2, Table 1). The $\delta^{13}C$ of non-encrusted *G. truncatulinoides* are also 0.5-1‰ lighter than their encrusted equivalents.
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Figure 4.2. Seasonal changes in $\delta^{18}$O and $\delta^{13}$C isotopes of G.ruber (p) (pink), O. universa (blue), G. truncatulinoides encrusted (black) and non-encrusted (green), with annual averages in a) $\delta^{18}$O calcification temperature (filled circles) calculated using $\delta^{18}$O seawater and matched to depth-adjusted hydrographic data shown with labelled depth horizons b) Dissolved inorganic carbon (DIC) (dark green line) and chlorophyll a (light green line) from 0-25m, compiled during the sediment trap deployments 1998-2000 and 2008-2010. Errors on measurements represent +/- $\sigma$.

4.5.2. Seasonal changes in trace element composition

Figures 4.3a-e show that Mg/Ca, Li/Ca of select species generally follow the seasonal changes in temperature and $[CO_3^{2-}]$ with higher (lower for Li/Ca) values coinciding with higher temperatures and $[CO_3^{2-}]$ in late summer. The Mg/Ca of O. universa has a weaker relationship with temperature and is positively offset from the other species by ~3
mmol/mol (Figure 4.3b). The Sr/Ca, U/Ca and B/Ca in *G. ruber* (p) and *O. universa* varies with higher temperatures and [CO$_3^{2-}$] but the range in Sr/Ca and U/Ca of especially non-encrusted *G. truncatulinoides*, is much larger than *G. ruber* (p) or *O. universa* and is offset to more positive values (Figure 4.3d-e). A large range of B/Ca values in *G. truncatulinoides* is equal to the combined range of *G. ruber* (p) and *O. universa* (~110 µmol/mol) (Figure 4.3f).

**Figure 4.3.** Seasonal changes in a) Mg/Ca for *G. ruber* (p) and *G. truncatulinoides* (encrusted and non-encrusted) represented by filled circles, b) Mg/Ca for *O. universa* only which is offset from other species. c) Li/Ca for *G. truncatulinoides* (non-encrusted) and *G. ruber* (p). d) Sr/Ca, e) U/Ca and f) B/Ca of all species studied. Seasonal changes in 0-25m (50-100m for *O. universa* only) temperature (red, dotted lines) and [CO$_3^{2-}$] (blue, dotted lines) are added for reference. The errors on measurements are Mg/Ca = +/- 2.26%, Li/Ca = +/- 2.39%, Sr/Ca = +/- 0.56%, U/Ca = +/- 1.79%, B/Ca = +/- 2.22% (according to Misra et al. 2014).
4.5.3. Controls on trace element incorporation

Table 4.2a-b displays the multiple linear regression (MLR) results. Calcification temperature explains most of the variance \( (r^2 = 0.69-0.89) \) in Mg/Ca, Li/Ca and Sr/Ca (Figures 4.4a-d, Table 4.2a). Salinity also explains a small amount of variance in Mg/Ca, (Table 4.2a), but when we added salinity-temperature residuals to the Mg/Ca-temperature model, there was no improvement in the slope coefficient (0.306) and only a slight improvement in the \( r^2 \) (from 0.87 to 0.88), suggesting any salinity influence on Mg/Ca is negligible, as expected from the small seasonal range at this site (Figure 4.1b). Although significant, calcification temperature only explains 22% of the variance in Cd/Ca and acts as a secondary control on B/Ca (Table 4.2a). This site has negligible dissolved phosphate present in surface waters so unfortunately there were not enough measurements at depth to test a Cd/Ca relationship with \([\text{PO}_4^{3-}]\) in planktonic foraminifera. Changes in area density/shell thickness explain most of the variation in Ba/Ca, Sr/Ca and U/Ca of G. truncatulinoides from this study \( (r^2 = 0.73-0.93) \) (Table 4.2b, Figure 4.5a-c) and also the B/Ca in G. ruber (p), O. universa, and non-encrusted G. truncatulinoides (Figure 4.5a). Li/Ca of non-encrusted G. truncatulinoides and G. ruber (p) display an inverse relationship with temperature but the large variability in O. universa and encrusted G. truncatulinoides show no significant relationship with temperature (Figure 4.4d).
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**Figure 4.4.** Geochemical temperature proxies

**a)** Multi-species Mg/Ca-temperature calibrations for *G. ruber* (p), *G. truncatulinoides* non-encrusted and encrusted from this study (filled circles), Anand et al. (2003) (open circles), and Regenberg et al. (2009) (crosses). Multi-species calibration lines for each are shown in the figure legend. **b)** Mg/Ca-temperature calibrations for *O. universa* from this study, Anand et al. (2003) and Lea et al. (1999). **c)** Separate Sr-temperature calibrations for non-glaborotaliid and *G. truncatulinoides* from this study (filled circles), Elderfield et al. (2000) (open squares), and Cleroux et al. (2008) (open diamonds). **d)** Li/Ca-temperature relationship for *G. ruber* (p) and *G. truncatulinoides* (non-encrusted). Error bars are as stated in Figure 4.3.
Even within our limited size fractions, we see an influence of test size on intraspecies variations in trace elements (Table 4.3). Higher B/Ca values weakly correlate with larger shells but only in G. ruber (p) ($r^2 = 0.44$) (Table 4.3), whereas higher U/Ca are strongly associated with larger shells in all species ($r^2 = 0.52-0.81$), except encrusted G. truncatulinoides (Figure 4.5d). Smaller non-encrusted G. truncatulinoides shells have significantly higher Mg/Ca but lower Sr/Ca ($r^2 = 0.71-0.81$), whereas smaller encrusted G. truncatulinoides have lower Mg/Ca ($r^2 = 0.30$) (Table 4.3). However, it is likely the changes of Mg/Ca in G. truncatulinoides are due to migration of individuals as they grow with larger individuals present at depth and smaller ones in the surface mixed layer (Figure 4.2a).

**Figure 4.5.** Linear regressions of area density with a) B/Ca for G. ruber (p) (pink dots) O. universa (blue dots), G. truncatulinoides non-encrusted (green dots). Encrusted G. truncatulinoides are shown in black but are not included in the regression. b) Sr/Ca and c) U/Ca of G. truncatulinoides non-encrusted/encrusted with area density. d) Shell size relationship with U/Ca in G. ruber (p), O. universa, G. truncatulinoides non-encrusted/encrusted. Errors on area density measurements are AD +/- (1/n), B/Ca = +/- 2.22%, Sr/Ca = 0.56%, U/Ca = 1.79%.
4.5.4. Trace element association

Figure 4.6a-c shows the results of PCA analysis on selected groups of species. Higher B/Ca, U/Ca, Sr/Ca, and Ba/Ca are associated with higher area densities, $[\text{CO}_3^{2-}]$ and calcification temperatures experienced by *G. ruber* (p) (Figure 4.6a). The corresponding multipanel scatterplot in Figure 4.6a support these observations because B/Ca is well correlated with U/Ca, Sr/Ca, and Ba/Ca. In both non-encrusted and encrusted *G. truncatulinoides*, higher Mg/Ca, Sr/Ca and U/Ca are strongly associated with higher calcification temperatures whereas B/Ca is not strongly associated with any environmental parameter (Figure 4.6b). Groupings of trace elements remain broadly similar when all species are combined, with higher Mg/Ca corresponding with lower Li/Ca (as seen previously in Figure 4.3a, c) and strong correlations between Sr/Ca and Ba/Ca (Figure 4.6c). Although Cd/Ca does not express strong significant relationships with any particular environmental parameter (e.g. in Table 4.2a, temperature only explains 22% of the variation in Cd/Ca), it does share a weak correlation with Mg/Ca in Figure 4.6a, c supporting the MLR result in Table 4.2a.
Figure 4.6. Principal component analyses diagrams and associated multipanel scatterplots to show the distribution of trace elements in a) *G. ruber* (p) (pink dots) and *O. universa* (blue dots) (specifically for Ba/Ca, Sr/Ca, U/Ca, Cd/Ca), b) *G. truncatulinoides* non-encrusted (green dots) and encrusted (black dots) (specifically for Mg/Ca, Ba/Ca, Sr/Ca, U/Ca, Cd/Ca) c) *G. ruber* (p), *O. universa*, *G. truncatulinoides* non-encrusted (specifically for B/Ca). The numbers in the lower panels of the scatterplot show Pearson correlation coefficients values, where the font size is proportional to its value.
### A. All species

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mg/Ca (G. ruber p, G. truncatulinoides all)</th>
<th>Li/Ca (G. ruber p, G. truncatulinoides-non-encrusted)</th>
<th>Ba/Ca (G. ruber p, O. universa)</th>
<th>Sr/Ca (G. ruber p, O. universa)</th>
<th>B/Ca (G. ruber p, O. universa, G. truncatulinoides non-encrusted)</th>
<th>U/Ca (G. ruber p, O. universa)</th>
<th>Cd/Ca (G. ruber p, O. universa)</th>
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<td>-0.0011</td>
<td>91.44***</td>
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<td>0.0063</td>
<td><strong>0.0113</strong>*</td>
<td><strong>3.581</strong>*</td>
<td>-0.268</td>
<td><strong>-0.014</strong>*</td>
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<tr>
<td>[CO₃²⁻]</td>
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<td>-0.044</td>
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<td>0.573</td>
<td><strong>0.169</strong></td>
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<td>17.41</td>
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<td>Adjusted r²</td>
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<td>0.69</td>
<td>0.74</td>
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</table>

### B. G. truncatulinoides (encrusted and non-encrusted)

<table>
<thead>
<tr>
<th>Factor</th>
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<th>Sr/Ca (G. truncatulinoides all)</th>
<th>U/Ca (G. truncatulinoides all)</th>
<th>Cd/Ca (G. truncatulinoides all)</th>
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<tbody>
<tr>
<td>Area density</td>
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<td><strong>-0.111</strong></td>
<td><strong>-7.854</strong></td>
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<tr>
<td>Size</td>
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<td><strong>0.029</strong></td>
<td>0.000066</td>
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<td>Calcification temperature</td>
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<td><strong>0.088</strong></td>
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<td>[CO₃²⁻]</td>
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<tr>
<td>Multiple r²</td>
<td>0.86</td>
<td>0.87</td>
<td>0.95</td>
<td>0.31</td>
</tr>
<tr>
<td>Adjusted r²</td>
<td>0.73</td>
<td>0.83</td>
<td>0.93</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Table 4.2. Tables showing the multiple linear regression results for A. a mixture of all species and B. G. truncatulinoides only (encrusted and non-encrusted). Species combinations were selected based on similar inter and intra-species trace element variations. Coefficients indicate the slope value or 'sensitivity' of the relationship between the variable and trace element ratio. Variables shown in bold significantly contribute to the model * p < 0.05, ** p < 0.001, *** p = 0. Adjusted r² accounts for sample size and number of independent variables.
## Intra-species size effect

<table>
<thead>
<tr>
<th>Trace element</th>
<th>G. ruber (p) (size range = 110 μm)</th>
<th>O. universa (size range = 135 μm)</th>
<th>G. truncatulinoides (non-encrusted) (size range = 326 μm)</th>
<th>G. truncatulinoides (encrusted) (size range = 396 μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg/Ca</td>
<td>-0.16</td>
<td>0.11</td>
<td><strong>-0.85</strong></td>
<td><strong>0.55</strong></td>
</tr>
<tr>
<td>Li/Ca</td>
<td>0.46</td>
<td>0.11</td>
<td>0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>Ba/Ca</td>
<td>0.22</td>
<td>0.12</td>
<td>0.27</td>
<td>0.38</td>
</tr>
<tr>
<td>Sr/Ca</td>
<td>-0.07</td>
<td>-0.44</td>
<td><strong>0.84</strong></td>
<td>0.14</td>
</tr>
<tr>
<td>B/Ca</td>
<td><strong>0.66</strong></td>
<td>-0.18</td>
<td>0.32</td>
<td>0.23</td>
</tr>
<tr>
<td>U/Ca</td>
<td><strong>0.72</strong></td>
<td><strong>0.76</strong></td>
<td><strong>0.90</strong></td>
<td>0.28</td>
</tr>
<tr>
<td>Cd/Ca</td>
<td>0</td>
<td>0.18</td>
<td>-0.72</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Table 4.3.** The Pearson's correlation coefficients ($R$) of trace element-shell size regressions of selected planktonic foraminifera species. Values in bold indicate significant correlations * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. 
4.6. Discussion

In the following discussion, we use the environmental differences expressed over a seasonal cycle and between our different species to explore the controls on trace element incorporation into foraminiferal calcite.

What controls variability in trace element concentrations?

4.6.1 Mg/Ca & Li/Ca

The Mg/Ca-temperature calibration based on *G. ruber* (p) and *G. truncatulinoides* agrees closely with previous multi-species temperature calibrations (Anand et al. 2003, Cléroux et al. 2008, Regenberg et al. 2009), but the Globorotaliid calibration from Regenberg et al. (2009) is positively offset (Figure 4.4a). Mg/Ca of *O. universa* from this study is lower compared to Lea et al. (1999) and shows less scatter compared to Anand et al. (2003). However, the *O. universa* Mg/Ca from this study is approximately half as sensitive to temperature than previous work (Lea et al. 1999, Anand et al. 2003). Less scatter in the Mg/Ca of *O. universa* could be due to selection of the Sargasso genotype only as opposed to mixed genotypes used in Anand et al. (2003) from the same study area.

Li/Ca decreases by 2-3% per °C increase in temperature for both *G. ruber* (p) and non-encrusted *G. truncatulinoides* which is in good agreement with previous Li-temperature sensitivities from benthic foraminifera and aragonite (Marriott et al. 2004a, b). This is supported by observations of higher Li/Ca in the last glacial (Hall and Chan, 2004a), consistent with greater uptake of Li under lower temperatures. We see no significant temperature effect and much larger variability in Li/Ca in *O. universa* and encrusted *G. truncatulinoides*. Some studies have suggested a [CO$_3^{2-}$] or calcification rate control on Li incorporation (Hall and Chan, 2004, Lear and Rosenthal, 2006, Ni et al. 2007), but we find no response of Li/Ca to changes in [CO$_3^{2-}$], shell area density or size, (Table 4.2a-b). In our analysis, Li/Ca is most inversely associated with Mg/Ca supporting a temperature
control and least associated with \([\text{CO}_3]^{2-}\), area density and size discounting a calcification rate control on seasonal timescales (Figure 4.6a,c). However, the less sensitive (and apparently species-specific) relationship of Li/Ca to temperature (2-3% per °C) compared to Mg/Ca (9-11% per °C) makes it a less attractive proxy for reconstructing past temperature changes.

### 4.6.2. Sr/Ca

Figure 4.4c shows that Sr/Ca in surface species increases by 1-2% per °C (Table 4.2a), which is comparable to the temperature sensitivity of Sr/Ca in O. universa in a previous culture study (Lea et al. 1999). Previous core-top studies have found no temperature influence in surface dwelling species compared to the Globorotaliids (Elderfield et al. 2000, Mortyn et al. 2005, Cleroux et al. 2008). Greater Sr incorporation in calcite has also been linked to faster calcification rate in inorganic experiments (Lorens, 1981, Paquette and Reeder, 1995) and this has been previously observed in coccolithophores (Rickaby et al. 2002, Stoll et al. 2002) and some planktonic foraminifera species (see Table 4.1 for references). As we have seen in the Chapter 3, temperature positively influences the calcification rate of foraminifera so it is difficult to discern the individual effects of these two variables on Sr incorporation. However, we find that Sr/Ca in surface species shares a stronger relationship with δ¹⁸O \((r^2 = 0.62)\) than area density \((r^2 = 0.39)\) or shell size (no significant relationship) suggesting temperature is in fact the dominant control. If Sr/Ca in G. truncatulinoides is controlled by calcification rate, as described by the MLR results in Table 4.2b, we would expect higher Sr/Ca in larger, thicker shells, but this is not the case (Figure 4.5b). This suggests that Sr/Ca in G. truncatulinoides could also reflect temperature changes, as supported by a good correlation with previous Sr/Ca-temperature relationships (Eelderfield et al. 2000, Cleroux et al. 2008) (Figure 4.4c) and Mg/Ca (Figure 4.6b). However, if the Sr of all species in this study were controlled by temperature, we would expect the surface dwelling species to have greater Sr/Ca than G. truncatulinoides, but they are depleted in Sr compared to G. truncatulinoides (Figure
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4.4c). This offset between surface and deeper dwellers could be explained by the Sr/Ca of seawater (Elderfield et al. 2000), which increases with depth, opposite to temperature effects (de Villiers et al. 1999). However, changes in Sr/Ca over the range of calcification depths (0-400m) would only account for a 0.01 mmol/mol increase in Sr/Ca from the calcification depths of surface to deeper dwellers at this site (de Villiers, 1999). Additionally changes in seawater Sr/Ca would not explain why non-encrusted G. truncatulinoides, some of which also calcify in the surface waters, are more enriched than G. ruber (p). Alternatively, differences in shell construction e.g. thick crust formation may affect the Sr partitioning in Globorotaliids (Mortyn et al. 2005, Cléroux et al. 2008). Therefore, we suggest that Sr/Ca in surface and deeper dwelling foraminifera primarily reflect temperature changes, but offset in Sr composition between the surface and deeper-dwelling species may reflect differing shell calcification processes, such as crust thickening. Similar to Li/Ca, Sr/Ca sensitivity to temperature is low (1-2% per °C) and only just larger than analytical error (0.56%), limiting its use as a palaeo-temperature proxy.

4.6.3. B/Ca & U/Ca

4.6.3.1. Calcification temperature effects

As suggested from Table 4.2a, some of the B/Ca variability could be explained by a temperature control on boron incorporation but the relationship between B/Ca and temperature is weak ($r^2 = 0.44$) and we find no significant relationship between B/Ca and Mg/Ca in G. ruber (p) and O. universa, suggesting any temperature effect is secondary (Figure 4.6a). This secondary temperature influence on B/Ca is supported by comparison with other studies where although B/Ca is generally higher in warmer temperatures, there is a lot of scatter (Figure 4.7a). U/Ca has no significant relationship with temperature (Table 4.2a) supported by its positive correlation with Li/Ca and inverse correlation with Mg/Ca (Figure 4.6c).
4.6.3.2. Carbonate chemistry effects

U/Ca and B/Ca have been previously suggested as proxies for the ocean carbonate system based on culture experiments (Sanyal et al. 1997, Allen et al. 2011; 2012) (see Table 4.1). Our results show that carbonate chemistry has no significant influence on B incorporation and only explains 38% of the variations in the U/Ca of G. ruber (p) and O. universa (Table 4.2a, Figure 4.7b). In O. universa, our seasonal range of 30 μmol/kg of [CO$_3^{2-}$] should be equivalent to a 0.7 nmol/mol change in U/Ca according to Russell et al. (2004) which is much smaller than the ~6 nmol/mol change we actually observe (Figure 4.3e). Instead, a lot of U/Ca variability can be explained through shell size fractionation in individual species (Table 4.3), which we will discuss in the next section. The 60-75 μmol/mol range in B/Ca in G. ruber (p) and O. universa should equate to between a ~300- >600 [CO$_3^{2-}$] μmol/kg according to culture calibrations on the same species (Allen et al. 2011; 2012), but they only undergo seasonal changes of 18-30 μmol/kg in [CO$_3^{2-}$] respectively (Figure 4.3f). Indeed, the full range in B/Ca recorded in this study (Figure 4.5a) should be equivalent to a pH range of ~0.6 according to culture calibrations (Allen et al. 2012) but the pH range is actually only 0.1 units over the depth habitats of all species.

Direct comparison with other core-top and sediment-trap based (i.e. non-culture studies) planktonic B/Ca-[CO$_3^{2-}$] records, show that our Sargasso Sea data has the largest range in B/Ca with one of the smallest ranges in [CO$_3^{2-}$] (Figure 4.7b). The lack of a distinct B/Ca-[CO$_3^{2-}$] relationship between different studies and species clearly indicate there are other competing controls on boron incorporation in the natural environment.

One control on B/Ca could be [B(OH)$_4$/HCO$_3$]$_{seawater}$ which is a function of temperature and pH (Allen et al. 2012). However, as previously shown at this site in G. ruber (white), the temperature and pH only cause negligible variations in [B(OH)$_4$/HCO$_3$]$_{sw}$ (Babila et al. 2014), equivalent to a 3-5 μmol/mol change in B/Ca according to calibrations from Allen et al. (2012), just a fraction of the total ~110 μmol/mol range observed in this study (Figure 4.7c).
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Fig. 4.7. B/Ca from this study and other planktonic species from Yu et al. (2007), (2013) and Foster, (2008) varying with a) Temperature b) $[\text{CO}_3^{2-}]$ and c) $[\text{B(OH)}_4/\text{HCO}_3]_{\text{seawater}}$

4.6.3.3. Calcification rate and shell size

We find that B/Ca displays a strong positive correlation with area density pointing to a calcification rate control on boron incorporation in G. ruber (p), O. universa and non-encrusted G. truncatulinoides (Fig. 4.5a). This is supported by recent inorganic precipitation experiments which have shown a precipitation-rate dependency on boron incorporation (Ruiz-Agudo et al. 2012, Uchikawa et al. 2015, Gabitov et al. 2014), suggesting that calcite growth rates have some involvement in boron partitioning. If thicker
shells represent faster calcite growth/calcification rates, as suggested by previous investigations (Spero et al. 1997, Bijma et al. 1999, Barker and Elderfield, 2002, this study), then this mechanism could explain why thicker shells with higher area densities contain more boron. This could also explain the apparent secondary temperature control on B/Ca as calcification rates increase at higher temperatures (Chapter 3).

We also find B/Ca has a weak correlation with *G. ruber* (p) shell size ($r^2 = 0.44$) and U/Ca has a strong positive correlation with shell size in all species ($r^2 = 0.51-0.81$) (Table 4.3, Figure 4.5d). This is consistent with previous observations of greater B/Ca in larger shells of *G. ruber* (white and pink) (Ni et al. 2007, Babila et al. 2014, Naik and Naidu 2014, Henehan et al. 2015) and U/Ca in *G. ruber* (white and pink) and *G. sacculifer* (Ni et al. 2007). Most studies have attributed this shell size fractionation to faster growth and hence calcification rates in larger individuals (Ni et al. 2007, Babila et al. 2014, Naik and Naidu 2014), which, similar to coccolithophores, may be less effective at discriminating against incorporation of trace elements when growth and calcification rates are higher, and hence they incorporate higher concentrations into the calcite lattice (Rickaby et al. 2002, Russell et al. 2004, Ni et al. 2007, Schmidt et al. 2008). However, if this were the case in all of our species, we would also expect to see a positive relationship between B/Ca and shell size in *O. universa* and non-encrusted *G. truncatulinoides* and also a positive relationship between area density and U/Ca, but we do not. Whilst assuming larger shells grow faster and hence have faster calcification rates is an inconsistent deduction, it may be true for at least some species, such as *G. ruber* (white and pink). For instance, Babila et al. (2014) observed a 15-20 µmol/kg offset in B/Ca between the 200-300 and 300-400 µm size fractions of *G. ruber* (white). Although they attribute this offset between size fractions to increased light intensity governing greater boron incorporation in larger shells due to a greater density of symbionts, we argue it could equally be due to increased growth/calcification rates in larger shells. We find that area densities in larger *G. ruber* (p) shells from this study are on average higher, compared to area densities from smaller shells; 311-363µm (equivalent sieve size = 250-300 µm, Chapter 3, Figure 3.1a) =
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1.23 x 10^{-4} \mu g/\mu m, 371-431 \mu m (equivalent sieve size = 300-355 \mu m) = 1.34 x 10^{-4} \mu g/\mu m.

We find this offset in area densities between larger and smaller shells of *G. ruber* (p) in which is equivalent to a \(-22 \mu mol/kg\) offset in B/Ca, almost the same discrepancy between sieve-size fractions observed by Babila et al. (2014). Yet Babila et al. (2014) discount a calcification rate control on B/Ca because they do not see a similar offset between size fractions for Sr/Ca and Mg/Ca. However, a calcification rate control would not necessarily have to be reflected in both Mg/Ca and Sr/Ca to be plausible in B/Ca. In this study, we see no correlation of area density with either Mg/Ca or Sr/Ca in surface dwelling species. Likewise, Ni et al. (2007) concluded there was no calcification control on Mg/Ca in *G. sacculifer* and *G. ruber* even though there was in B/Ca, Li/Ca and U/Ca. We discount a light intensity control on B incorporation in this study because we see no correlation between shell size and $\delta^{13}C$ in *G. ruber* (p) (which would be enhanced in larger shells with more symbiont activity due to more carbon fixation, Spero and Parker, 1985). Additionally, we also see no effect of shell size on boron incorporation in symbiont-bearing *O. universa*. Finally, non-encrusted *G. truncatulinoides* has a greater B/Ca concentration than *O. universa*, and comparable B/Ca to *G. ruber* (p), which should not be the case if its incorporation were primarily controlled by symbionts enhancing intracellular pH.

Recently, [PO$_4^{3-}$] was proposed as a B/Ca control because it can interact with the calcite lattice, forming amorphous calcium carbonate allowing for greater incorporation of boron (Henehan et al. 2015). However, [PO$_4^{3-}$] at this site is negligible in the surface waters and only reaches 0.26 \mu mol/kg at \(-400m\), which according to the B/Ca-[PO$_4^{3-}$] relationship described by Henehan et al. (2015), should be equivalent to \(-30 \mu mol/mol\) change in B/Ca, just a quarter of our observed 110 \mu mol/mol range. Higher B/Ca present in [PO$_4^{3-}$]-depleted surface waters (*G. ruber* (p) and non-encrusted *G. truncatulinoides*) at this site further disproves a dominant [PO$_4^{3-}$] control. We suggest that these correlations between higher B/Ca in *G. ruber* (w) and [PO$_4^{3-}$] may also be caused by higher growth and calcification rates in areas of higher productivity. This is supported by observations of larger *G. ruber* (w) shells containing greater B/Ca during upwelling periods (Naik and...
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Naidu, 2014) and is also consistent with higher B/Ca observed in cultured foraminifera fed every day compared to open-ocean foraminifera from a comparable pH (Henehan et al. 2015).

Contrary to shell size fractionation of B/Ca in just G. ruber (p), U/Ca demonstrates strong species-specific, shell-size fractionations in all species. U/Ca increases relatively more in larger shells of symbiont-bearing species (G. ruber (p) and O. universa) than non-symbiont G. truncatulinoides (Figure 4.5d). Our results strongly suggest a biological control on U incorporation, which is supported by the observed offset between foraminiferal partition coefficients (Russell et al. 2004) and laboratory-derived partition coefficients for inorganic calcite in seawater (Meece and Benninger, 1993). Calcification rate is unlikely to control U/Ca variability in surface species because it does not share a positive correlation with area density (Table 4.2a). Additionally, U/Ca shares an inverse relationship with [CO$_3^{2-}$] (Russell et al. 2004) and because shells calcify faster in higher [CO$_3^{2-}$] conditions (Bijma et al. 1999, Barker and Elderfield, 2002), it would be counter-intuitive for an increase in U/Ca to be attributed to faster calcification as suggested by Ni et al. (2007). Our results suggest that the incorporation of B is distinctly different from U, and that species-specific foraminiferal ‘vital effects’ exert considerably more control on the incorporation of U.

Overall, our results suggest more research is needed to quantify the impacts of calcification and biological fractionation on the incorporation of B and U before they can be used effectively as carbonate system proxies.

4.6.4. Crust and size control in G. truncatulinoides

In general, our non-encrusted G. truncatulinoides have higher concentrations of all trace elements except B/Ca, which also appears in equal concentrations in both non-encrusted and encrusted individuals (Figure 4.5a). This indicates that the addition of secondary crust, which is formed in deeper waters, does not significantly affect the bulk shell B/Ca, 106
unlike for other trace elements such as Mg/Ca, Ba/Ca, Sr/Ca, U/Ca (Figure 4.3a, d, e, Table 4.2b). For instance, the B/Ca of encrusted individuals could conceivably reflect the area density from the non-encrusted stage, but the addition of the crust offsets this relationship (Figure 4.5a). Unlike their non-encrusted equivalents, there is a large range of B/Ca of encrusted _G. truncatulinoides_; The 100 µmol/mol range of B/Ca in encrusted individuals almost covers the entire 110 µmol/mol range of B/Ca in non-encrusted, _O. universa_ and _G. ruber_ (pink) combined (Figure 4.5a). Other encrusted Globorotaliid species, such as _G. inflata_ and _G. scitula_ and also _O. universa_ have shown large intratest B/Ca variability (Hathorne et al. 2009, Allen et al. 2011), even when grown under identical conditions (Allen et al. 2011) suggesting this heterogeneity reflects changes in the microenvironment, rather than an external environmental control. This is further supported by the lack of association of B/Ca with other environmentally controlled trace elements e.g. Mg/Ca (Figure 4.6b).
4.7. Conclusions

This study presents geochemical measurements (Mg/Ca, Li/Ca, Sr/Ca, B/Ca, U/Ca, δ¹⁸O and δ¹³C) of the planktonic foraminifera species *G. ruber* (p), *O. universa*, and *G. truncatulinoides* (non-encrusted and encrusted), with associated shell flux, size and thickness measurements, in context with measured hydrographic data. This combined approach allows us to identify primary environmental, seawater chemistry and ecological controls on these geochemical proxies. Our main findings are as follows:

1. Mg/Ca, Sr/Ca and Li/Ca are dominantly controlled by calcification temperature in selected species. Our overall Mg/Ca-temperature relationship agrees well with previous studies showing a 9-11% change in Mg/Ca per °C change in temperature, but only ~5% change in Mg/Ca per °C change in temperature is observed for *O. universa* Sargasso genotype. The low sensitivities of Sr/Ca and Li/Ca to temperature (1-2% per °C and ~3% per °C respectively) limit their use as temperature proxies in planktonic foraminifera.

2. B/Ca has no significant relationship with carbonate system parameters in this study or within inter-study comparison. Instead, we suggest that boron incorporation may be controlled by calcification rate due to a strong positive correlation with shell area density (thickness)/calcification rate, and a weak shell size dependence in *G. ruber* (p). In *G. truncatulinoides*, addition of the secondary crust formed in deeper waters does not significantly affect the bulk shell B/Ca, but may contribute to the heterogeneity observed between samples.

3. Whilst we find a weak relationship of U/Ca with the carbonate system in surface species, there are significant increases of U/Ca with shell size in all species, suggesting a strong biological control on U/Ca.

4. Our results suggest selecting specimens from a limited shell thickness, as well as shell size range is required in order to determine the dominant environmental controls on trace element incorporation in planktonic foraminifera.
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Contributions

K. S. selected the sampling periods, collected the data and interpreted results. P.J. aided K.S. in performing PCA analysis, P. A. conceived the project idea, and P.A. and P.F.S. secured funding. M. C. provided the sediment trap samples. K.S., P.A., & P.F.S. contributed their relevant expertise to constructing the manuscript.
Jane Goodall (1931- )

“What you do makes a difference, and you have to decide what kind of difference you want to make”
Chapter 5: Inhibition of planktonic foraminifera calcification

5. Inhibition of calcification in subtropical planktonic foraminifera during the industrial era

This Chapter has been submitted to Nature Geoscience, June 2015:


5.1 Abstract

Invasion of anthropogenic carbon dioxide (CO$_2$) into our oceans is causing an ongoing reduction in calcium carbonate saturation that is predicted to make it difficult for crucial marine plankton groups such as coccolithophores, pteropods and foraminifera to sustain their calcium carbonate exoskeletons (Orr et al. 2005, Moy et al. 2009, Lischka et al. 2011). Yet attempts to evaluate the impact on calcification (Moy et al. 2009, de Moel et al. 2009) have been hindered by an inability to directly compare the calcification capability of today’s plankton species with their counterparts from exclusively pre-industrial times. Here we compare an exceptional and unique collection of strictly pre-industrial planktonic foraminifera collected by the Challenger Expedition in 1875 (prior to significant oceanic CO$_2$ invasion) with the same foraminifera species from the industrial-age collected by sediment traps between 1998 and 2010. We present data on foraminifera calcification from the subtropical North Atlantic, an oceanic region that has witnessed a disproportionately high degree of atmospheric CO$_2$ invasion relative to its area (Sabine et al. 2004). We measured shell area densities (a proxy for calcite shell thickness, Marshall et al. 2013) of two species (Orbulina universa and Globorotalia inflata) with contrasting ecologies (Hemleben et al. 1989) and find that their industrial-age shells from the years 1998 to 2010 are, respectively, 23% and 17% thinner than their pre-industrial counterparts. These findings are remarkably close to the predicted effects on calcification of ocean acidification arising from the observed uptake of at least 25% of all
anthropogenic CO₂ into the subtropical North Atlantic (Takahashi et al. 2009) and validate laboratory predictions for decreased calcification ability under these conditions (Bijma et al. 1999). Because foraminifera can contribute up to ~40% of the total vertical carbonate flux in this region (Salmon et al. 2015), our findings may portend a major on-going reduction in this surface-to-deep ocean carbonate 'pump'.

5.2 Main Text

The ocean has absorbed a third of the anthropogenic carbon dioxide (CO₂) released during the industrial era (Khatiwala et al. 2009) which is predicted to cause reductions in surface ocean pH, carbonate ion concentration ([CO₃²⁻]) and calcium carbonate saturation at a rate unparalleled in the past 300 million years (Hönisch et al. 2012, Bijma et al. 2013). Laboratory experiments (Bijma et al. 1999) and marine chemical models (Caldeira and Wickett, 2003, Orr et al. 2005) have predicted that reduced surface ocean carbonate saturation may make it harder for an array of important marine organisms to secrete their calcium carbonate shells and exoskeletons (Orr et al. 2005, Moy et al. 2009, Lischka et al. 2011). Some recent studies (Moy et al. 2009, de Moel et al. 2009) have attempted to compare the calcification ability of a specific plankton species today with their pre-industrial counterparts (before accelerated CO₂ emissions) by using today's seafloor surface sediments to represent 'pre-industrial' (pre-1875) times. However, today's seafloor surface sediments inevitably also incorporate microfossils from the modern industrial era that could lead to circularity when comparing microfossil data from these surface sediments to modern sediment traps or plankton tows. We circumvent this circularity by utilising an exceptional and unique collection of planktonic foraminifera obtained in the year 1875 before the exponential rise of anthropogenic CO₂ emissions in the industrial era. This material, obtained by the Challenger Expedition (which laid the foundations of oceanography), was collected from the Sargasso Sea in the subtropical North Atlantic Ocean (Figure 5.1a,b) and represents an archive of pre-industrial plankton microfossils uncontaminated by modern individuals from the industrial era.
Chapter 5: Inhibition of planktonic foraminifera calcification

The subtropical North Atlantic Ocean is of profound importance for assessing the impact of changing ocean chemistry on plankton calcification because it has witnessed a disproportionately high degree of anthropogenic CO₂ invasion relative to its area (~25% of all anthropogenic CO₂ invasion into the global ocean (Sabine et al. 2004, Takahashi et al. 2009), Figure 5.1a) for two primary reasons. First, the Revelle factor (defined as the ratio of the fractional change in seawater CO₂ to the fractional change in seawater dissolved inorganic carbon, Revelle and Suess, 1957) in these subtropical waters is unusually low, giving a higher oceanic buffer capacity, which has enabled increased absorption of anthropogenic CO₂ (Sabine et al. 2004) (Figure 5.1a). Second, entrainment of CO₂ during the formation of subtropical mode water (STMW) (Bates, 2012) encourages transport of anthropogenic CO₂ into the interior of the subtropical Atlantic. This means that the subtropical Atlantic has not only absorbed anthropogenic CO₂ at a rate twice as fast as expected from surface waters in equilibrium with increasing atmospheric CO₂ concentrations (Bates et al. 2002), but 70% of all dissolved CO₂ in sub-surface STMW is now of anthropogenic origin (Bates, 2012). This makes the Sargasso Sea, in the core of the subtropical Atlantic, an ideal location to monitor the calcification response of planktonic foraminifera to anthropogenic CO₂ invasion. Although higher latitudes such as the Southern Ocean have been suggested to provide a 'bellwether' for the impact of CO₂ invasion on biocalcification (Fabry et al. 2009), their comparatively higher Revelle factors result in a disproportionately lower degree of CO₂ invasion relative to their area (Sabine et al. 2004).
Figure 5.1 a) Location of the study area in the Sargasso Sea (yellow cross) relative to the respective subsurface (50m) anthropogenic CO₂ concentration in the North Atlantic Ocean, b) Bathymetric map of the Oceanic Flux Program (OFP) sediment-trap (modern), Surface (recent) sediment and 'pre-industrial' (Challenger Expedition, 1875) surface sediment samples in relation to Bermuda island. 29 bi-weekly sediment trap time-series (modern) samples collected from 1500m traps (31°50'N, 64°10' W), 3 (recent) surface sediments collected from ~4500m (between 31°39'-31°54', 64°04'-64°15') and 4 pre-industrial surface sediments collected from between ~3000-4800m (32A-32°1°N, 64°51°W, 35C-32°15°N, 65°8°W, 57B-32°9°N, 65°10°50', 37-32°18°N, 65°38°W). Anthropogenic CO₂ concentrations in map (a) were obtained from the GLobal Ocean Data Analysis Project (GLODAP) and mapped using Ocean Data View. Bathymetry contours in map (b) are modified from the General Bathymetric Chart of the Oceans data set.
This study compares the calcification response of two species of planktonic foraminifera (*Orbulina universa*, *Globorotalia inflata*) from pre-industrial to industrial times (Figure 5.2a,b) using sample archives consisting of a historical collection of four pre-industrial surface sediments (pre-1875), three recent seafloor surface sediments collected ~120 years after the pre-industrial sediments (age: ~1920-1990, Supplementary Discuss. S1), and twenty-nine modern-day sediment-trap samples (age: 1998-2000 and 2008-2010) directly overlying the recent seafloor surface sediments (Figure 5.1b, Appendix 4 Data D1). All sediment samples are from the same oceanographic setting (Figure 5.1a, Supplementary Tables S1 & S2) and lie above the calcite saturation horizon (Feely et al. 2004). Two species were selected that have contrasting ecologies and depth habitats in this region in order to monitor calcification changes with depth: *O. universa* inhabits the upper 100 m of the photic zone, whereas *G. inflata* inhabits the STMW (100-450 m) (Anand et al. 2003).

To estimate the calcification response of planktonic foraminifera, we use shell area density (a proxy for shell thickness), where higher area densities represent thicker, and therefore more heavily calcified, shells (Marshall et al. 2013). We combine the area densities for all modern sediment trap (1998-2010), recent (1920-1990) and pre-industrial (pre-1875) samples to provide an estimate of the mean calcification response for these respective time periods (see Supplementary Discuss. S1). We use flux weighted mean area densities to represent the shell thicknesses for *O. universa* and *G. inflata* (Appendix 4, Data D1), which show a significant reduction (*p << 0.05*) in modern (1998-2010) samples in comparison to ‘pre-industrial’ samples (pre-1875) (Figures 5.2a-2b) (Supplementary Tables S1 & S2). For photic zone dwelling *O. universa*, shell area densities for recent (~1920-1990) and modern (1998-2010) samples were 14% and 23% lower (i.e. thinner), respectively, than their pre-industrial counterparts (pre-1875) (*p << 0.05*) (Figure 5.2a). Modern (2008-2010) *G. inflata* shells were ~17% thinner than their pre-industrial equivalents (*p << 0.05*), although recent (1920-1990) *G. inflata* shells were statistically indistinguishable from their pre-industrial equivalents (Figure 5.2b). *G. inflata*
shell area densities from 2008-2010 were also 4% lower (thinner) than their equivalents from 1998-2000 ($p << 0.05$) (Figure 5.2b), in contrast to modern *O. universa* shells that remained statistically similar throughout the decade from 1998 to 2010 ($p = 0.08$) (Figure 5.2a).

Figure 5.2. Average area densities through time (year) for a) *Orbulina universa* and b) *Globorotalia inflata* from the >125μm size fraction for ‘pre-industrial’ surface sediment, recent surface sediment, and all modern 1500m sediment-trap samples. For *O. universa* and *G. inflata* respectively: Pre-industrial (pre-1875) ($n = 87, n = 191$), recent surface sediment (~1920-1990) ($n = 77, n = 87$) and flux weighted average area densities were utilised for modern sediment-trap samples 1998-2000 ($n = 167, n = 65$) and 2008-2010 ($n = 156, n = 127$) (see Appendix 4, Data D1). Y-axis error bars denote the ± 95% confidence interval on area density (see Supplementary Tables S1-S2) and X-axis error bars denote the error on age estimates. Please note that ages for pre-industrial samples represent the upper age limit as defined by the year of collection. Ages shown in figure are: ‘pre-industrial’ shells (1875), recent sediment shells (1920-1990, average = 1955- see Supp. Disc. S1 for more details) and sediment trap shells (1998-2000, average = 1999, and 2008-2010, average = 2009)

Because no statistically significant difference exists between *O. universa* area densities from 1998-2000 and 2008-2010, we combined flux-weighted averages of the two time periods to represent modern area densities of this species. When evaluating the range of pre-industrial versus modern area densities for both species, we find that both time intervals yielded shells with comparatively lower area densities (thin shells), but the pre-industrial interval predominantly yields shells of higher area densities (thick shells) (Supplementary Figure 1a-b), in accordance with observations from core-top sediments in
the Southern Ocean (Moy et al. 2009). These findings suggest that the reduction in average shell thickness in modern foraminifera is partly driven by the presence of fewer thicker (more heavily calcified) shells, rather than solely a greater abundance of thinner (more lightly calcified) shells. Nevertheless, we do find that both species also show a trend towards proportionally greater numbers of thinner shells in modern samples compared to those from pre-industrial or recent seafloor sediments (Supplementary figures 1a-b), supporting an inhibition of calcification at both ends of the shell thickness spectrum during the industrial era.

To determine whether the observed decreases in shell thickness between pre-industrial and modern shells are driven by a reduction in calcification arising from anthropogenic ocean acidification, we first consider other potentially confounding possibilities. First, we find two cryptic morphotypes of *O. universa* in the Sargasso Sea: the thinner ‘Sargasso’ type and thicker ‘Caribbean’ type (de Vargas et al. 1999, Morard et al. 2009). However, we use only the thinner Sargasso morphotype (Supplementary figure 2a) because it is more abundant in our study area and can potentially be more sensitive to changes in ocean chemistry owing to its thinner calcium carbonate wall. This eliminates the possibility of the observed shell thickness changes being driven by a shift in the relative abundance of cryptic morphotypes with contrasting shell thicknesses. Second, we utilise only clean specimens of both species from exceptionally well-preserved samples (Supplementary Discuss. S2). All specimens selected for shell measurements were transparent (i.e. well preserved) with negligible overgrowths/crust formation for *G. inflata*, providing consistency in our area density measurements between different samples (Supplementary figure 2a-b). Third, no significant difference exists in the planktonic foraminifera species assemblages between the sediment-trap and seafloor surface sediments (*p* >> 0.1), suggesting that the water column assemblage is accurately represented in the seafloor sediments and no significant change in oceanographic regime has occurred. The lack of shell fragmentation and presence of the thinner-walled, transparent, *O. universa* morphotype (‘Sargasso-type’) in all sediment samples are
indicative of no post-depositional alteration, especially in the seafloor sediment samples. Given the lack of contribution from any of these possibilities, we thus interpret the trend towards decreasing shell area densities (thinner shells) in our time series for both species (Figure 5.2a-b) to be a gradual calcification response to changing ocean chemistry imparted by anthropogenic CO₂ invasion (Figure 5.1a).

We now evaluate our findings against the expected response of foraminifera calcification given that [CO₃²⁻] at this location has decreased by ~18 and ~20 µmol kg⁻¹ in the surface water and STMW respectively since the mid-1980s (Bates, 2012, Bates et al. 2012). For approximately the same time period in our dataset (i.e. from ~1920-1990 versus modern), we observe a ~13-15% reduction in shell calcification for *O. universa* and *G. inflata* (Figure 5.3). Yet the relationship between shell area density and [CO₃²⁻] from the same biweekly sediment trap time series (Supplementary Discuss. S3a) predicts a larger ~21% reduction in *O. universa* calcification in response to the ~18 µmol kg⁻¹ reduction in surface [CO₃²⁻] since the mid 1980s (Bates et al. 2012) (Supplementary Discuss. S3b). The observed calcification reduction in our dataset (i.e. 13-15%, from ~1920-1990 to modern) therefore underestimates the expected impact on calcification in comparison to the sediment trap-based calibration (i.e. 21%, from the mid 1980s to modern) by about 6-8% (Supplementary Discuss. S3b). One explanation for this discrepancy is that, in contrast to the calcification reduction caused by decreasing [CO₃²⁻], rising temperatures have previously been shown to promote calcification and thereby mitigate the impacts of ocean acidification (Manno et al. 2012). Although the observed sea surface temperature warming of ~0.33°C since 1983 (Bates et al. 2012) would only counterbalance ~1-2% of the calcification reduction in *O. universa* estimated from measured [CO₃²⁻] since the mid 1980s, a larger sea surface temperature warming of 1°C during the past century (Bates et al. 2012) could have offset the calcification reduction observed between the recent sediments (1920-1990) and modern samples by ~5% (i.e. potentially accounting for most of our 6-10% discrepancy) (Supplementary Discuss. S3c). Higher temperatures could also explain the increase in the average size of *O. universa* shells during the industrial era as
shown by the offsets through time in the slope of the relationship between shell length and shell weight (Supplementary Figure 2a).

![Graph showing percentage decrease in calcification relative to a 'pre-industrial' baseline for O. universa and G. inflata.](image)

**Figure 5.3. Percentage decrease in calcification relative to a ‘pre-industrial’ baseline for O. universa and G. inflata.** All percentage changes were calculated relative to the ‘pre-industrial’ baseline area density. When compared to ‘pre-industrial’ surface sediment, shell area density of G. inflata is not significantly different in the recent surface sediment, ~13% and ~17% lower in shells from 1998-2000 and 2008-2010 sediment traps respectively. Shell area density of O. universa is ~14% lower in recent surface sediment and ~23% lower in modern samples (1998-2010 sediment traps) than ‘pre-industrial’ surface sediment.

Our observation that O. universa shells become progressively larger but thinner through the industrial era (Supplementary Figure 2a; i.e. for a given shell weight, shells are becoming larger, thus they must also becoming thinner) suggests that as well as lower [CO$_3^{2-}$] driving a thinning of shells, increasing temperatures may have simultaneously allowed this species to construct larger shells (Caron et al. 1987). Alternatively, larger shell sizes could have evolved to reduce the surface area:volume ratio for effective calcification, or to increase buoyancy (Caromel et al. 2014) in warmer industrial-age waters. Considering our sediment trap relationships for area density with both [CO$_3^{2-}$] and temperature together (Supplementary Discuss. S3a), we estimate a ~24 μmol/kg
reduction in subtropical subsurface $[\text{CO}_3^{2-}]$ in the last century based on observed area
densities of the pre-industrial versus modern samples for $O. \text{universa}$ (Supplementary
Discuss. S3c). This estimated $\sim 24 \mu\text{mol/kg}$ reduction in subtropical subsurface $[\text{CO}_3^{2-}]$
since the pre-industrial is in line with expected surface ocean $[\text{CO}_3^{2-}]$ reduction, which
ranges between 18 $\mu\text{mol/kg}$-29 $\mu\text{mol/kg}$ from polar to tropical regions respectively (Orr et
al. 2005).

$G. \text{inflata}$ calcifies in the STMW (Hemleben et al. 1989, Anand et al. 2003) that
experiences higher accumulation rates of anthropogenic CO$_2$ than the upper thermocline
(Bates et al. 2002) where $O. \text{universa}$ calcifies (Anand et al. 2003). This may explain why
$G. \text{inflata}$ has also undergone a similar proportional reduction in calcification to $O. \text{universa}$ from recent (1920-1990; surface sediments) to modern (1998-2010; sediment traps) (Figure 5.3). Yet most of the reduced calcification in $G. \text{inflata}$ occurred in the last
30-90 years with no statistically significant change between pre-industrial (pre-1875) and
recent times (1920-1990) (Figure 5.2b). This delayed inhibition of calcification in $G. \text{inflata}$ to
anthropogenic CO$_2$ invasion suggests that either its physiology may allow it to
withstand a higher threshold of CO$_2$ invasion than $O. \text{universa}$, or that it reflects the
observed later invasion and subsequent faster accumulation of CO$_2$ into STMW in
comparison to surface waters, especially since the 1980s (Bates, 2012). The faster, early
decline in calcification of $O. \text{universa}$ (Sargasso type) suggests that its photic zone habitat
conferred greater susceptibility to the anthropogenic CO$_2$ invasion that initially penetrated
the surface ocean photic zone, compared to the deeper STMW habitat of $G. \text{inflata}$. The
response of both species to lower $[\text{CO}_3^{2-}]$ appears to be similar between recent (1920-
1990) and modern shells as indicated by comparable reductions in calcification (13-15%,
Figure 5.3).

By comparing shell calcification of modern planktonic foraminifera species with their
counterparts from exclusively pre-industrial times (thereby precluding contamination of
‘pre-industrial’ samples with modern ‘industrial-age’ foraminifera), our study provides the
first unambiguous test of the calcification response of planktonic foraminifera to
anthropogenic CO₂ release during the industrial era. We find 17-23% reductions in calcite shell thicknesses (area densities), and thus reduced calcification, in *O. universa* and *G. inflata*. Furthermore, the time-evolving patterns of calcification inhibition in *G. inflata* and *O. universa* are mainly governed by both the respective evolution of [CO₃²⁻] concentrations of the water masses that these species inhabit and possibly also by species ecological traits. A rapid reduction in calcification of *G. inflata* since the 1920-1990s reveals the importance of monitoring sub-surface species dwelling in ocean interior water masses such as STMW, as their sub-surface CO₂ inventories are increasing at a rate faster than in overlying surface waters (Bates et al. 2002). This could mean that species inhabiting these depths may be more susceptible to decreases in [CO₃²⁻]. If our observed 17-23% reduction in shell calcification of two species of planktonic foraminifera (with contrasting ecologies and depth habitats) from the subtropical Atlantic is indicative of a wider ongoing inhibition of shell calcification in the planktonic foraminifera, our findings may portend the early stages of a major decrease in surface-to-deep ocean calcium carbonate export, with profound implications for global carbon cycling and marine ecosystem functioning. Since the majority of organic carbon is carried in association with calcium carbonate (Klaas and Archer, 2002), we also suggest a reduction in calcium carbonate export from the surface ocean could reduce the efficiency of the organic carbon pump, decreasing the future capacity of the oceans to absorb anthropogenic atmospheric CO₂.
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Contributions

K. S. collected the data and interpreted initial results. P. A. conceived the project idea, sample selection and funding. M. C. provided the sediment trap and recent samples. K.S., P.A and P.F.S. constructed the manuscript and all authors contributed their relevant expertise to improve the final manuscript.
Supplementary information for “Inhibition of calcification in subtropical planktonic foraminifera during the industrial era”

Methodology
Sediment trap samples were picked from a 60% split in the 125-500, 500-1000 µm size fractions, which were processed after collection (Conte et al. 2001). Washed surface sediment and pre-industrial samples were picked (~30 individual shells) for *O. universa* and *G. inflata* after splitting the sample (>125µm size fraction) into a representative sub-sample i.e. such as used for faunal assemblage work in micropalaeontology (see Appendix 4, Data D2 for more details) (*O. universa*: $n = \text{min. 12, max. 52, avg. 33, G. inflata: } n = \text{min. 26, max. 80, avg. 42}$). Each batch of whole foraminifera shells were ultrasonically cleaned in ultra-pure water and oven dried at 50-60°C.

Area density
Size-normalised weight was originally introduced by Lohmann (1995) and used by Broecker and Clark (2001) as a means to measure bottom water saturation state related to post-mortem dissolution (thinning) of shells. Dissolution is unlikely to have affected the planktonic foraminifer shells from surface sediments here because they lie above the calcite saturation horizon (Feely et al. 2004) and shells were exceptionally well preserved i.e. transparent, with few fragments present (Appendix 4, Supp. Disc. S2). Consequent studies also have since shown that the shell size-normalised weights (indication of shell thickness) responded directly to $[\text{CO}_3^{2-}]$ (Bijma et al. 2002, Barker and Elderfield, 2002). However, these previous studies used a method of normalising foraminifera shell weights to the size by sieving shells into different size ‘windows’. This sieve-size based method for size-normalisation of foraminifera weights has since been modified to a method that measures the individual shell dimensions such as weight and area to obtain ‘area density’
(Beer et al. 2010, Marshall et al. 2013). For this 'area density' method, the lengths and areas of individual shells were obtained using ImageJ (Schneider et al. 2012), after orienting shells with the aperture side facing upwards, following calibration using a microscale. Individual shells were weighed on a microbalance (precision = 1μg) and area densities were calculated by dividing the individual shell weight by the individual shell area (μg/μm²). These individual area densities were then averaged for each sample. Shell measurements were conducted on all shells (>125 μm) from the sub-samples, providing a representative estimation of shell thickness within the species population (Appendix 4, Data D2). Flux-weighted area densities for sediment trap samples were calculated from 1998-2000, 2008-2010 for O. universa and G. inflata by combining average area densities for each sample from the 125-1000μm size fraction, with their respective fluxes (tests/m²/day) (see Appendix 4, Data D1). The Sargasso morphotype of O. universa (de Vargas et al. 1999, Darling and Wade, 2008, Morard et al. 2009) was used in this study and distinguished from the Caribbean morphotype by identifying its characteristically thinner shells and lower pore densities under high magnification when picking (Morard et al. 2009).
Supplementary Discussion S1: Recent and pre-industrial surface sediment ages

Three recent surface sediment samples (1920-1990) used in this study were collected by a Van Veen Grab to capture the sediment water interface primarily for studying surface sediment lipid composition (not used here). Van Veen grab sampling minimally disturbs, and effectively captures, the topmost layer of sediment. These recent surface sediment samples (Appendix 4, Data D2) are unsuitable for radiocarbon measurements because during industrialisation, the increased combustion of $^{14}$C-depleted fossil fuels lead to the gradual decline in atmospheric $^{14}$C values, producing a dilution in radiocarbon signal and hence a lengthy plateau in the calibration curve, termed the 'Suess Effect' (Suess, 1955). As a consequence, calibrating radiocarbon ages of samples since ~1890 at the start of industrialisation can produce large potential age ranges (Keigwin and Guilderson, 2009). We are unable to $^{14}$C date the pre-industrial surface sediments as they are unique and irreplaceable samples, part of a historical collection of huge significance currently on loan from the Natural History Museum, London. $^{14}$C dating currently requires ~ 10-20 mg of foraminiferal material, which is equivalent to destroying ~660-1300 shells, (assuming an average foraminifera shell weighs approximately 15 µg), which would consume a major portion of the sample coarse fraction (on average < 1g).

Therefore, in order to calculate the age of our surface sediments, we compared sedimentation rates from two studies in the same vicinity as our study area (Keigwin and Jones, 1989, Haidar et al. 2000). We use the sedimentation rate of the closest sediment core (31°45’N, 64°21’W) to our surface sediment samples, which is based on assuming continuous sediment deposition between 0.5-12.5 cm depth. This calculated sedimentation rate of 0.02 cm/yr is also consistent with the Holocene sedimentation rate from another nearby core from the NE Bermuda Rise, ~33°42’N, 57°37’W (Keigwin and Jones, 1989, Keigwin and Boyle 1999). Assuming stable sedimentation at the OFP
sediment trap site throughout the past century, the estimated age range of our recent sediment samples (Van Veen Grab) between 0.25-1.5 cm depth is ~12 to 75 years older than the year of the sample collection (1997-1999), equating to 1923-1986, if bioturbation is absent. However, all Van Veen samples used in this study were collected from well-oxygenated sediments, and have likely experienced some bioturbation. This means the top few centimetres represent mostly modern foraminifera, as indicated by zero or negative radiocarbon ages, such as from the nearby Bermuda Rise surface sediments (Keigwin, 1996). These surface sediments (Van Veen Grab) have also experienced up to 111 more years of deposition than the Challenger surface sediment samples (collected in 1875), underscoring the fact that these Van Veen Grab surface sediments are younger than the Challenger pre-industrial sediments.

Surface sediments were taken from 0.25-0.5 cm and 0.5-1.5 cm depth because the surface sample from 0-0.25cm was depleted for a previous study. Area density measurements for both *Globorotalia inflata* and *Orbulina universa* are respectively grouped for these two surface sediment depths (0.25-0.5 cm and 0.5-1.5 cm), since there are no statistically significant differences between area density measurements from these two depths, as expected of bioturbated sediment layers (Keigwin, 1996).
Supplementary Discussion S2: Light microscope images for both species a) *Orbulina universa* and b) *Globorotalia inflata* demonstrate the exceptional preservation of 'transparent' shells through time. Modern samples are from the Oceanic Flux Program 1500m sediment trap, 31°50'N, 64°10' W, recent surface sediment shells collected at ~4500m are from 31°39'-31°54' N, 64°04'-64°15' W and pre-industrial surface sediment shells were collected between ~3000-4800m from 32°18'0"N, 65°38'0"W (see Chapter 5, Figure 1).
Supplementary Discussion S3: Application of the *O. universa* sediment-trap shell area density calibrations with \([\text{CO}_3^{2-}]\) and temperature, to estimate \([\text{CO}_3^{2-}]\) and calcification changes since recent (1920-1990) and pre-industrial (pre-1875) sediment samples.

**S3a. Calibration construction**

Area density-\([\text{CO}_3^{2-}]\) and area density-temperature calibrations were constructed using both *O. universa* and *G. ruber* (pink) from modern sediment traps (1998-2000 and 2008-2010) in the Sargasso Sea (Oceanic Flux Program, 31°50' N, 64°10'W) by combining shell measurements with hydrographic data collected from the nearby Bermuda Atlantic Time Series (31°40' N, 64°10'W). We used both *G. ruber* (pink) \((n = 6)\) with *O. universa* \((n = 15)\) in the same calibration in order to extend the range in \([\text{CO}_3^{2-}]\) and temperature as governed by the subsurface depth habitat of *O. universa*, and the surface mixed layer habitat of *G. ruber*, (pink). Both species were combined in two linear calibrations for \([\text{CO}_3^{2-}]\) and temperature: Area density \((1 \times 10^{-4} \ \text{µg/µm}^2) = -2.17 + (0.0135 \times [\text{CO}_3^{2-}])\) and Area density \((1 \times 10^{-4} \ \text{µg/µm}^2) = -0.026 + (0.043 \times \text{Temperature})\), where larger area densities (thicker shells) are synchronous with higher \([\text{CO}_3^{2-}]\) and temperatures. We used these equations to predict expected changes in area density in context with \([\text{CO}_3^{2-}]\) and temperature changes from pre-industrial (pre-1875) and recent (1920-1990) to modern (1998-2010).

**S3b. Application to recent (1920-1990) sediments**

From the \(\delta^{18}\text{O}\)-derived calcification temperatures of our sediment trap *O. universa* shells (1998-2000 and 2008-2010), we observe that present-day *O. universa* lives ~40-100m which is equivalent to calcification at ~ 227 µmol/kg \([\text{CO}_3^{2-}]\) and temperature ~ 21.14°C. Our sediment-trap calibrations show that the reported 17.5 µmol/kg decrease in surface...
water \([\text{CO}_3^2-]\) and 0.33°C increase in temperature since 1983 (Bates et al. 2012) would be equivalent to a predicted net change in \(O. \text{universa}\) area density from 1.13 \(\mu\text{g/\mu m}^2\) to 0.89 \(\mu\text{g/\mu m}^2\), or a 21% reduction, (with temperature accounting for ~1-2% of this change alone). However, subsurface changes in \([\text{CO}_3^2-]\) and temperature associated with the depth habitat (40-100m) of \(O. \text{universa}\) may be slightly less than the surface changes. This is consistent with the observation that \(O. \text{universa}\) area density from the equivalent time period in this study reflects a decrease from 1.02 \(\mu\text{g/\mu m}^2\) in recent (1920-1990) sediments to on average 0.91 \(\mu\text{g/\mu m}^2\) in modern sediment-traps (1998-2010) (Supplementary Table S1), which is a 11% decrease (and equivalent to a -8 \(\mu\text{mol/kg}\) change in \([\text{CO}_3^2-]\) using our sediment trap calibration). Given that surface \([\text{CO}_3^2-]\) at this site decreases by -0.58 \(\mu\text{mol kg yr}^{-1}\) (Bates et al. 2012), a -8 \(\mu\text{mol/kg}\) change in \([\text{CO}_3^2-]\) since 1998-2010 would suggest recent (1920-1990) shells \(O. \text{universa}\) shells actually calcified between 1984-1996, falling within the upper part of this estimated age bracket. This narrow age is estimated within the assumption that subsurface \([\text{CO}_3^2-]\) within the depth habitat of \(O. \text{universa}\) (40-100m) has decreased at the same rate as surface \([\text{CO}_3^2-]\) since 1983. However, if subsurface \([\text{CO}_3^2-]\) has decreased at a slower rate than surface \([\text{CO}_3^2-]\) since 1983, the estimation of recent surface sediment age could be slightly older.

**S3c. Application to pre-industrial (pre-1875) sediments**

Temperatures in the upper 400m at BATS have increased at a rate of 0.01°C/yr (Joyce et al. 1999, Bates et al. 2012) equivalent to a 1°C increase in \(O. \text{universa}\) calcification temperatures over the past century from 20.14°C in pre-industrial (~1875) to the observed 21.14°C in modern (1998-2010). Using the sediment-trap calibration, we estimate a 1°C increase in calcification temperatures from pre-industrial to modern is equivalent to a 6% increase in \(O. \text{universa}\) area density of 0.05 \(\mu\text{g/\mu m}^2\). Supplementary Table 1 shows that observed \(O. \text{universa}\) area density decreases from an average of 1.18 \(\mu\text{g/\mu m}^2\) in pre-industrial (pre-1875) samples to 0.896 \(\mu\text{g/\mu m}^2\) in modern samples (2008-2010), a total change of 0.28 \(\mu\text{g/\mu m}^2\). Without a 0.05 \(\mu\text{g/\mu m}^2\) increase in area density due to a 1°C
temperature rise in the last century, *O. universa* area density could potentially have decreased by a total of 0.33 μg/μm² solely due to decreasing [CO$_3^{2-}$] which, according to our area density-[CO$_3^{2-}$] calibration, is equivalent to a reduction in subsurface [CO$_3^{2-}$] of ~25 μmol/kg since the pre-industrial. This estimated ~25 μmol/kg reduction in subtropical subsurface [CO$_3^{2-}$] since the pre-industrial is in line with expected surface ocean [CO$_3^{2-}$] reduction, which range between 18 μmol/kg-29 μmol/kg from polar to tropical regions respectively (Orr et al. 2005).
### Supplementary Table S1. Average area densities of sediment trap and surface sediment samples for *Orbulina universa*.

<table>
<thead>
<tr>
<th>Sample-set (collection date*)</th>
<th>Age</th>
<th>Latitude (°N)</th>
<th>Longitude (°W)</th>
<th>Water Depth (m)</th>
<th>Total shells measured (n)</th>
<th>Flux-weighted area density of <em>O. universa</em> ((1 \times 10^{-4} \mu g/\mu m^2)) ± 95% C.I. ((1 \times 10^{-4})) **</th>
<th>± Standard Error ((1 \times 10^{-4})) ***</th>
<th>σ ((1 \times 10^{-4}))</th>
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<td>156</td>
<td>0.896 ± 0.029</td>
<td>0.00149</td>
<td>0.186</td>
<td>Oceanic Flux Program</td>
</tr>
<tr>
<td>Sediment-trap 1998-2000</td>
<td>1998-2000</td>
<td>31° 50'</td>
<td>64° 10'</td>
<td>1500</td>
<td>167</td>
<td>0.924 ± 0.042</td>
<td>0.00212</td>
<td>0.274</td>
<td>Oceanic Flux Program</td>
</tr>
<tr>
<td>Recent surface sediment</td>
<td>~1920-1990</td>
<td>Between 31°39'-31°54'</td>
<td>64° 04'</td>
<td>~4500</td>
<td>77</td>
<td>1.02 ± 0.046</td>
<td>0.013</td>
<td>0.196</td>
<td>Oceanic Flux Program</td>
</tr>
<tr>
<td>'Pre-Industrial' Challenger</td>
<td>Pre-1875</td>
<td>32°1'-32°17'</td>
<td>64°50'-65°7'</td>
<td>~3000-4800m</td>
<td>87</td>
<td>1.18 ± 0.072</td>
<td>0.036</td>
<td>0.333</td>
<td>Natural History Museum, London</td>
</tr>
</tbody>
</table>

* For more information on collection dates, please see Appendix 4 Data D1-D2. ** Confidence intervals calculated using the formula \(\bar{x} ± t \frac{\sigma}{\sqrt{n}}\), where \(\bar{x}\) = Mean area density, \(t\) = multiplier (≈2 for 95% confidence), \(\sigma\) = 1 standard deviation, \(n\) = number of measurements per sample. *** Error = mean area density × \(\frac{1}{n}\).
Supplementary Table S2. Average area densities of shells from sediment trap and surface sediment for *Globo
talia inflata*.

<table>
<thead>
<tr>
<th>Sample-set (collection date) *</th>
<th>Age</th>
<th>Latitude (°N)</th>
<th>Longitude (°W)</th>
<th>Water Depth (m)</th>
<th>Total shells measured (n)</th>
<th>Flux-weighted area density of G. <em>inflata</em> (1 x 10⁻⁴ µg/µm²)</th>
<th>± 95% C.I. (1 x 10⁻⁴) **</th>
<th>± Standard Error (1 x 10⁻⁴) ***</th>
<th>σ (1 x 10⁻⁴)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment-trap 2008-2010</td>
<td>2008-2010</td>
<td>31° 50'</td>
<td>64° 10'</td>
<td>1500</td>
<td>127</td>
<td>2.02</td>
<td>0.057</td>
<td>0.016</td>
<td>0.315</td>
<td>Oceanic Flux Program</td>
</tr>
<tr>
<td>Sediment-trap 1998-2000</td>
<td>1998-2000</td>
<td>31° 50'</td>
<td>64° 10'</td>
<td>1500</td>
<td>65</td>
<td>2.10</td>
<td>0.049</td>
<td>0.031</td>
<td>0.204</td>
<td>Oceanic Flux Program</td>
</tr>
<tr>
<td>Recent surface sediment</td>
<td>~1920-1990</td>
<td>Between 31°39'-31°54'</td>
<td>64° 04'</td>
<td>~4500</td>
<td>87</td>
<td>2.37</td>
<td>0.086</td>
<td>0.027</td>
<td>0.399</td>
<td>Oceanic Flux Program</td>
</tr>
<tr>
<td>'Pre-Industrial' Challenger surface sediments (1875)</td>
<td>Pre-1875</td>
<td>32°1'-32°17'</td>
<td>64°50'-65°7'</td>
<td>~3000-4800m</td>
<td>191</td>
<td>2.42</td>
<td>0.069</td>
<td>0.027</td>
<td>0.475</td>
<td>Natural History Museum, London</td>
</tr>
</tbody>
</table>

* For more information on collection dates, please see Appendix 4 Data D1-D2. ** Confidence intervals calculated using the formula \( \bar{x} \pm t \frac{\sigma}{\sqrt{n}} \), where \( \bar{x} \) = Mean area density, \( t \) = multiplier (= ~2 for 95% confidence), \( \sigma \) = 1 standard deviation, \( n \) = number of measurements per sample. *** Error = mean area density x ( \( \frac{1}{n} \)).
Supplementary Figure 1. Distribution of area density measurements for a) *Orbulina universa* and b) *Globorotalia inflata* showing shell thickness (area density) variability within modern sediment-trap shells (light grey), surface sediments (medium grey) and pre-industrial age surface sediment (dark grey). The largest discrepancy between the sample-sets occurs at higher area densities (thicker shells).
Supplementary Figure 2. Shell weight-length relationship for all the shells used in this study of a) *O. universa* and b) *G. inflata*. The Shell weight-length relationship has comparable slope gradients for each sample-set for *O. universa* (Shell length (µm) = 554 + 4.78 × shell weight (µg) for 1998-2010, Shell length (µm) = 490 + 5.29 × shell weight (µg) for 1920-1990, Shell length (µm) = 463 + 4.78 × shell weight (µg) for pre-1875) suggesting that only one morphotype is represented (Marshall et al. 2015). The offset of each regression line at the intercept for *O. universa* sample-sets is caused by a progressive increase in size from pre-industrial to modern shells. If calcite crust had added significant weight to *G. inflata*, we would expect the shell weight-length graph to flatten off at higher shell weight end with a larger unit weight per unit of size.
6. Conclusions

6.1 Addressing the aims

In this study, I set out to understand the controls and interactions of environmental and ecological variables on planktonic foraminifera (PF) flux, shell parameters and trace element incorporation, and to apply this new understanding to assess if calcification of planktonic foraminifera has been affected by anthropogenic ocean acidification. The four research aims outlined in the Introduction (section 1.5) have been addressed as follows:

1. **What physical/chemical/biological variables primarily control the species flux and growth of planktonic foraminifera?** Through comparison of seasonal and interannual variations of PF flux, I have established the depth and dynamics of the mixed layer is the primary control on total PF flux at this site, because it determines the abundance and availability of their food phytoplankton (section 2.7.1-2.7.2). I also showed that transient eddies amplify the seasonal flux of especially deeper-dwelling species (i.e. *G. truncatulinoides, G. hirsuta*) through mixing of the water column, which aids their annual reproductive migration in addition to increasing food supply (section 2.7.3). As PF flux makes up to 40% of total carbonate flux in this region, (with deeper-dwelling species making up the majority 60-90% of this), then factors which affect mixed layer dynamics such as the North Atlantic Oscillation could also affect the PF flux and therefore carbonate ‘pump’ (section 2.8). Salmon et al. 2015, *Biogeosciences*.

2. **What controls the shell parameters and calcification of planktonic foraminifera?** I have used an effective size-normalisation procedure to establish area densities (µg/µm²) of individual shells (proxy for shell thickness) (Figure 3.2). I show that [CO₃²⁻] and temperature are the dominant controls on shell thickness and hence calcification of *G. ruber (p)* and *O. universa* (section 3.5.1.1). I find no
evidence for shell calcification being controlled by optimum growth conditions (measured using species flux) but I do observe higher fluxes of smaller, thicker *O. universa* shells during a cyclonic eddy (section 3.5.1.2). The change in shell thickness prior to size change suggests that shells may alter their size to control their buoyancy in the water column. The different sensitivities of area density to 

\[ [\text{CO}_3^{2-}] \]

seen in culture vs. open-ocean studies may be due to morphotype-specific differences between *O. universa* Sargasso type used in this study and the Caribbean type used culture (section 3.5.2). Alternatively, the synergistic effects of temperature and 

\[ [\text{CO}_3^{2-}] \]

may have a greater influence on planktonic foraminifera in the open-ocean, compared to published culture work. This chapter will be submitted to *Paleoceanography*.

3. **What controls the trace element composition in planktonic foraminifera?** I find species growth and calcification processes play an important role in controlling trace element incorporation in planktonic foraminifera. I suggest calcification rates (inferred from shell thickness) primarily control boron incorporation in planktonic foraminifera because I find a strong positive correlation between area density (shell thickness) and B/Ca (section 4.5.3, figure 4.5a). Likewise, I find strong species-specific relationships between U/Ca and shell size suggesting a biological, rather than calcification rate control on U incorporation in to PF shells (section 4.5.3, figure 4.5d). Although Mg/Ca, Sr/Ca and Li/Ca reflect temperature, I find that Sr/Ca and Li/Ca are not sensitive enough to be used as temperature proxies in PF (section 4.6.1-4.6.2), and specific morphotype selection in *O. universa* will improve temperature estimates obtained from Mg/Ca (figure 4.4a). This chapter will be submitted to *Earth and Planetary Science Letters*.

4. **Has recent ocean acidification affected the calcification of modern planktonic foraminifera?** Yes. I find a 17-23% reduction in the shell thickness and hence calcification of *G. inflata* and *O. universa* respectively (selected for
different ecologies) from surface sediment samples collected in 1875 to sediment-trap samples collected between 1998-2010. By applying the area density-[CO$_3^{2-}$] and temperature calibrations developed in Chapter 3 to _O. universa_ area densities in this study, I find that the 23% reduction in calcification of _O. universa_ is equivalent to a ~24 μmol/kg reduction in [CO$_3^{2-}$] since 'pre-industrial' (pre-1875) which is in line with model-predicted reductions ranging between 18 μmol/kg-29 μmol/kg from polar to tropical regions respectively (Orr et al. 2005). Salmon et al. 2015, submitted to *Nature Communications*.

### 6.2 Implications and further research

The most salient findings from this study are visually synthesised in Figure 6.1. Questions remaining to be addressed are represented as letters as follows:

- **A-** What processes regulate the foraminiferal component of the carbonate pump on interannual timescales and how will ocean acidification affect it? (section 6.2.1)
- **B-** Why do discrepancies remain between biogeochemical calibrations constructed in open-ocean vs. culture environments and how can this be resolved? (section 6.2.2)
- **C-** How ubiquitous are morphotype-specific relationships in biogeochemical calibrations constructed using planktonic foraminiferal shell morphology and geochemistry? (section 6.2.3)
- **D-** Is there a reliable proxy for optimum growth conditions that can be detected in fossil foraminifera assemblages? (section 6.2.4)
- **E-** To what extent does growth influence shell parameters and hence trace element incorporation in planktonic foraminifera? (section 6.2.5)

Here I discuss how these overarching issues that could be addressed through further research.
Figure 6.1. Schematic summarising the interactions between the main processes affecting the use of biogeochemical proxies in planktonic foraminifera. Numbers correspond to the relevant chapter for discussion and letters correspond to further questions highlighted by this research.
6.2.1 Planktonic foraminifera carbonate ‘pump’

This research has shown that long-term climatic change has the potential to affect the carbonate pump in the oligotrophic North Atlantic through 1) The North Atlantic Oscillation (NAO) influence on mixed layer dynamics which consequently affects the total PF flux and therefore carbonate flux (Salmon et al. 2015) 2) Anthropogenic ocean acidification alters the ocean carbonate chemistry, reducing the calcification of planktonic foraminifera (Chapter 5), and consequently their component of total carbonate flux. Although I have theorised the causes behind long-term changes in carbonate flux, more research is needed to confirm this in the geologic record. Further high-resolution analysis of PF flux on decadal-centennial timescales would help to quantify their response to any changes in the NAO. As I have shown deeper-dwelling species make up the majority (60-90%) of the PF carbonate flux (Salmon et al. 2015), more work is needed to investigate how anthropogenic ocean acidification will affect these sub-surface dwelling species with generally much denser tests. Monitoring the response of sub-surface dwelling species to ocean acidification is especially important in areas such as the Sargasso Sea where the subsurface (subtropical mode water) experiences higher accumulation rates of anthropogenic CO₂ than the surface ocean (Bates et al. 2002).

6.2.2 Culture vs. open-ocean discrepancies

This research has highlighted the discrepancy between planktonic foraminifera shell calcification/trace element composition with environmental variables in culture and open-ocean studies. For instance, in Chapter 3 I showed that shell thicknesses of planktonic foraminifera grown in culture appeared much less sensitive to changes in carbonate chemistry compared to their open-ocean equivalents (section 3.5.2). Likewise, in Chapter 4, the Mg/Ca-temperature relationship for *O. universa*-Sargasso type from this study was ~50% less sensitive than that measured in culture (figure 4.4b) (Lea et al. 1999), whilst the B/Ca range in this study for *G. ruber* (p) and *O. universa* was much larger i.e. more sensitive, than could be explained solely by changes in [CO₃²⁻] measured on the same
species in culture (figure 4.7a) (Allen et al. 2011, 2012). I suggest that the sensitivity discrepancy between geochemical proxies measured in culture vs. open-ocean planktonic foraminifera could be due to the synergic effects of multiple co-varying environmental variables in open-ocean studies. More culture experiments are needed to test the combined effects of multi-stressor environments on calcification in order to effectively simulate the response of open-ocean environments to future climatic change i.e. decreasing carbonate ion concentration in tandem with increasing temperatures. Alternatively, different morphotypes could contribute to some discrepancies observed between open-ocean and culture studies (please see next section).

6.2.3 Morphotype-specific relationships

This study demonstrates the importance of separating species morphotypes when possible. For instance I observe less scatter in the Mg/Ca-temperature relationship for Sargasso-type O. universa than a previous study (figure 4.4b), which used mixed morphotypes of this species (Anand et al. 2003). Equally, morphotype-specific responses could explain the disparity between culture and open-ocean studies on the calcification responses of O. universa (Chapter 3) and between O. universa Mg/Ca-temperature relationships (Chapter 4), because culture work tends to use the Caribbean morphotype (Bijma et al. 1999, 2002, Russell et al. 2004, Allen et al. 2011) whilst open-ocean sites are characterised by a mixture of three morphotypes (de Vargas et al. 1999, Darling and Wade, 2008). Developing an understanding of morphotype-specific biogeochemical responses to environmental parameters for some species may resolve part of the variability observed between studies and ultimately demonstrate to what extent morphotypic variability affects the use of geochemical proxies in planktonic foraminifera.

6.2.4 Redefining optimum growth conditions

I show that planktonic foraminifera shells are not necessarily larger during higher fluxes (Salmon et al. 2015, Figures 3.9c-3.10c), suggesting that shell abundance and size are
not always reliable indicators of optimum growth conditions as previously thought (Schmidt et al. 2004a). In Chapter 4, section 4.6.3.3. I showed that growth of foraminifera detected through changes in shell size could be a determining factor of trace element concentration. It is thus critically important that future work concentrates on modelling the interaction between measured growth rates (culture work), abundances, and size for different planktonic foraminifera species will help identify an appropriate optimum growth proxy for use in the fossil record.

6.2.5 Shell parameters influence geochemical proxies

I show that changes in both shell size and thickness may affect the incorporation of trace elements differently (section 4.6.3.3) whereas previously, only changes in shell size have been thought to affect trace element composition of planktonic foraminifera. These results demonstrate the importance of measuring both shell thickness and size, (measured shell size, rather than sieve size), when interpreting the palaeo-record because they may represent different cellular processes, have different effects on geochemical proxies, and thus yield different information on seawater properties and processes. Further work should concentrate on gaining a better understanding about which cell processes determine shell size and thickness in planktonic foraminifera. Also, further research is needed to quantify the interactions and mechanism between growth rate on calcification in order to understand the extent of biological processes on calcite precipitation and thus shell geochemistry.
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