# The Influence of the Environment on Shell Morphology and Calcification in Planktonic Foraminifera

**Dissertation**
der Mathematisch–Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
Dipl.-Geol. Manuel Fritz Gerhard Weinkauf
aus Rüdersdorf bei Berlin

Tübingen
2014

Gedruckt mit Genehmigung der Mathematisch–Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

| | |
|---|---|
| Tag der mündlichen Qualifikation: | 06. 02. 2015 |
| Dekan: | Prof. Dr. Wolfgang Rosenstiel |
| 1. Berichterstatter: | Prof. Dr. Michal Kučera |
| 2. Berichterstatter: | Prof. Dr. Heinz-R. Köhler |

# The Influence of the Environment on Shell Morphology and Calcification in Planktonic Foraminifera

*Manuel F. G. Weinkauf*

*Ph.D. thesis*

2014

Eberhard–Karls-Universität Tübingen/MARUM Bremen

Eberhard–Karls-Universität Tübingen
Mathematisch–Naturwissenschaftliche Fakultät
Hölderlinstraße 12
72074 Tübingen
Germany

*and*

MARUM Bremen
Leobener Straße
28359 Bremen
Germany

Ph.D. thesis by Dipl.-Geol. Manuel F. G. Weinkauf
Day of submission: 3 December 2014
Day of oral presentation: 6 February 2015

Supervisor: Prof. Dr Michal Kučera
Second supervisor: Prof. Dr Heinz-R. Köhler
Head of Promotionsverbund: Prof. Dr Katja Tielbörger

Typeset in LaTeX with Palatino Linotype, Helvetica, and Letter Gothic

*If I have seen further it is by standing on ye sholders of Giants*

Sir Isaac Newton

# Acknowledgements

The work presented in this thesis has been conducted at the Eberhard–Karls University Tübingen and the MARUM (University Bremen) as part of the Minigraduierten-Kolleg 'Morphological Variability of Organisms under Environmental Stress', and I want to thank everyone from those institutes who was involved in this work in any way.

First and foremost, I want to thank Michal Kučera for the chance to be a member of his workgroup and for his great supervision during my Ph.D. studies. Furthermore I would like to thank my second supervisor, Heinz-R. Köhler, and the head of the Minigraduierten-Kolleg, Katja Tielbörger.

Further thank goes to James Nebelsick and Oliver Friedrich (Ruprecht–Karls University Heidelberg) for volunteering as members of my defence committee.

I gratefully acknowledge the valuable help of all the colleagues, TA's, HIWI's, and so on both at the University Tübingen and the MARUM Bremen who helped with sample preparation and administrative questions, and who always selflessly provided their advise on several occasions. Thanks to Philipp Munz, Hartmut Schulz, Margret Bayer, Birgit Lübben, Ute Wolpmann, Michael Siccha, Ralf Aurahs, Andreia Rebotim, Christiane Schmidt, Raphaël Morard, and all the others. Further thank goes to Agnes Weiner, Yvonne Milker, Anne Mohrholz, and René Hoffmann for check-reading this work and helping out with loads of suggestions and comments; and the other members of the Minigraduierten-Kolleg (Anne Mohrholz, Diedrich Sievers, Leonie Hannig, and Betty Springer) for several nicely spent evenings. I thank all my co-authors (Tobias Moller, Mirjam Koch, José Kunze, Kerstin Braun, and Fabian Bonitz) for their help in conducting the studies for this thesis and writing the related manuscripts, and all those people who were involved in obtaining the samples those works are based on (especially Hartmut Schulz, Joanna Waniek, and the captain and crew of the research vessel Meteor).

My special appreciation goes to my friends who had a great share in making this thesis possible and who made the time I spent on this project especially enjoyable: Yvonne (for great chats and her lovely dry humour), Agnes (for countless advices,

lots of friendly laughs, and an occasional kick up the backside whenever I needed it), Anne (for a warm place to sleep and great company on more than one occasion), Steffanie (principially the best HIWI one could imagine; and see, this time I wrote your name correctly), DD (for lovely discussions and for always selflessly helping out to build up a hangover for the next day), Leonie (for often being much more amusing than she realizes herself), and Dani, Markus, Kai, René, Reikja and Mario for chats, fun, and exploring the world together.

My very special thank goes to my family, for their invaluable support during all that time, without which nothing of this would have been possible.

My project was financed by the Ministerium für Wissenschaft, Forschung und Kunst Baden–Württemberg (LGFG stipend) and the J. M. Resig Fellowship of the Cushman Foundation, whose contributions are thankfully acknowledged.

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*Status: published*

Own contributions:

| | |
|---|---|
| Idea/Hypothesis | 60 per cent |
| Data acquisition | 70 per cent |
| Interpretation | 70 per cent |
| Writing | 60 per cent |

**Manuscript 2 (p. 81):** Weinkauf, M. F. G., Kunze, J. G., and Kučera, M. (in preparation) Seasonal variation in shell calcification of planktonic Foraminifera in the NE Atlantic reveals species-specific response to temperature, productivity, and optimum growth conditions, *Biogeosciences*

*Status: in preparation*

Own contributions:

| | |
|---|---|
| Idea/Hypothesis | 60 per cent |
| Data acquisition | 10 per cent |
| Interpretation | 60 per cent |
| Writing | 80 per cent |

**Manuscript 3 (p. 105):** Weinkauf, M. F. G., Moller, T., Koch, M. C., and Kučera, M. (2014) Disruptive selection and bet-hedging in planktonic Foraminifera: Shell morphology as predictor of extinctions, *Frontiers in Ecology and Evolution* 2: Article 64, doi:10.3389/fevo.2014.00064

*Status: published*

Own contributions:

| | |
|---|---|
| Idea/Hypothesis | 80 per cent |
| Data acquisition | 70 per cent |
| Interpretation | 80 per cent |
| Writing | 70 per cent |

**Manuscript 4 (p. 119):** Weinkauf, M. F. G., Braun, K., Bonitz, F., and Kučera, M. (in preparation) Stabilization and disruption as indicator of terminal stress and extinction in planktonic Foraminifera, *PLOS ONE*

*Status: in preparation*

Own contributions:

| | |
|---|---|
| Idea/Hypothesis | 80 per cent |
| Data acquisition | 80 per cent |
| Interpretation | 80 per cent |
| Writing | 80 per cent |

## Abstract

Understanding the effect of environmental stress on the morphology of a population can be developed into a versatile tool to reconstruct stress levels. Such knowledge could help to reconstruct past environments and to predict the state of a population, including future extinction. Especially for the latter aspect, morphometrics could be a valuable alternative for population-dynamics approaches, which suffer from the naturally high variability of population sizes. Calcitic marine microplankton, such as planktonic Foraminifera, offers an excellent model system for such studies. Planktonic Foraminifera occur in high abundances in the fossil record and their chambered shells allow the reconstruction of individual morphologies during their entire ontogeny. Their excellent fossilisation potential further allows to study natural experiments, which occurred over ecologically effective timescales that would have been impossible to simulate during laboratory experiments.

Planktonic Foraminifera have already been broadly applied for geochemical and population studies to reconstruct past environments. Their morphology and shell calcification have in contrast been subject to comparably few studies so far. This is unfortunate, since both parameters could be useful for past environmental reconstructions, recent environmental monitoring, and phylogenetic research. Since planktonic Foraminifera have a large share on the worldwide marine calcite deposition, environmentally induced changes in their shell calcification could furthermore significantly influence the oceanic carbon pump. This study therefore aims at a better understanding of the influence of changing environments, including results of environmental stress, on the biometry of planktonic Foraminifera. For this purpose, several foraminiferal species were investigated within three selected environmental settings: two Pleistocene sediment cores and one sediment trap series. The shell calcification intensity and morphology have been investigated in light of their relation to environmental forcing and biological stress.

The shell calcification intensity (amount of calcite present in the adult shell) shows signs of a universal positive correlation with carbonate saturation of the sea water. When the carbonate saturation is kept nearly constant, however, it is evident that shell calcification intensity is also influenced by other factors like temperature and productivity. Those secondary influences act species-specific and are presumably able to mediate or modify the effects of carbonate saturation. It could further be shown that cryptic speciation is a severe problem for calcification studies, because shell calcification is already significantly different between pseudo-cryptic species that have been commonly pooled together in the past. Shell size was in no case related to species abundance, what would have been expected under the assumption that species are most abundant under optimal environmental conditions. Together with the fact that shell calcification intensity is also variably correlated to species abundance, this implies that either species abundance is no versatile proxy for optimal growth conditions, or that optimal conditions are not uniformly related to biometric traits. Other phenotypic traits were observed to show characteristic deviations in relationship to environmental stress. The observed trends all led to a clear change in population morphology over ecologically relevant timescales as result of natural selective patterns. In a community which is exposed to near-lethal stress levels, this can culminate in a unique morphology that is clearly different from that of a less stressed population.

The obtained results imply that foraminiferal biometry, despite their unicellular level of organisation, reacts in complex ways toward changes in the environmental setting. Those reactions are complicated by the interplay of abiotic (environment) and biotic (stress) factors and the presence of hidden diversity. Further research is needed to minimize those problems.

## Kurzfassung

Ein genaueres Verständnis darüber, auf welche Weise Umweltstress die Morphologie einer Population beeinflusst, könnte sich als wertvolles Werkzeug für die Rekonstruktion vergangener Stress-Intensitäten und Umweltbedingungen herausstellen. Insbesondere könnte es hilfreich sein, den Zustand einer Population (inkl. der Vorhersage von Aussterbeereignissen) zu bestimmen. Morphometrische Studien eignen sich hier besser als Populations-Dynamik Ansätze, da letztere von den natürlicherweise großen Schwankungen der Populationsgröße beeinflusst werden. Kalzitisches marines Mikroplankton (z. B. planktonische Foraminiferen) sind ein ideales Modellsystem für solche Studien, da sie in hohen Häufigkeiten im fossilen Befund erhalten bleiben und ihre gekammerte Schale eine Rekonstruktion der gesamten Ontogenie zulässt. Ihr hervorragendes Fossilisationspotential erlaubt außerdem natürliche Experimente auf ökologisch wirksamen Zeitskalen zu untersuchen, die nicht im Labor simuliert werden könnten.

Planktonische Foraminiferen werden bereits häufig für geochemische und Populations-Studien verwendet, um vergangene Umweltbedingungen zu rekonstruieren. Ihre Schalen-Morphologie und -Kalzifikation wurden jedoch bisher selten untersucht, obwohl sie potentiell nützlich sind um vergangene Umweltbedingungen und Foraminiferen-Phylogenie zu rekonstruieren und rezente Ökosysteme zu monitorieren. Durch ihren hohen Anteil an der weltweiten Karbonat-Produktion könnte eine umweltbedingte Änderung ihrer Schalen-Kalzifikation zudem das ozeanische Karbonatsystem stören. Diese Studie versucht daher den Einfluss von Umweltänderungen (inkl. Stress) auf die Biometrie von Foraminiferen zu untersuchen. Zu diesem Zweck wurden mehrere Foraminiferen-Arten aus zwei pleistozänen Sedimentkernen und einer Sediment-Fallenserie bezüglich des Umwelteinflusses auf deren Morphologie und Schalen-Kalzifikation untersucht.

Die Kalzifikations-Intensität (Menge an vorhandenem Kalzit) ist generell positiv mit der Karbonat-Sättigung des Meerwassers korreliert. Unter konstanter Karbonat-Sättigung zeigen sich jedoch außerdem Spezies-spezifische Einflüsse von Temperatur und Produktivität auf die Kalzifikations-Intensität der Schalen, welche den Einfluss der Karbonat-Sättigung auf diesen Parameter vermutlich zu jeder Zeit modifizieren. Kryptische Speziation stellt zudem ein signifikantes Problem für Kalzifikationsstudien dar, da die Schalen-Kalzifikation auch zw. kryptischen Spezies die traditionell oft zusammengefasst wurden deutlich unterschiedlich ist. Die Schalen-Größe war in keinem Fall mit der Spezies-Häufigkeit korreliert, was man erwartet hätte, wenn Letztere ein Maß für optimale Umweltbedingungen wäre. Zudem zeigt auch die Schalen-Kalzifikation unterschiedliche Korrelationen mit der Spezies-Häufigkeit, so dass entweder die Spezies-Häufigkeit kein brauchbarer Indikator für optimale Umweltbedingungen ist, oder dass optimale Bedingungen die Schalen-Biometrie von Foraminiferen nicht einheitlich beeinflussen. Andere morphologische Parameter zeigten charakteristische Änderungen welche auf Umwelt-Stress zurückzuführen sind. Diese Trends resultierten sämtlich in deutlichen Änderungen der Populations-Morphologie, ausgelöst durch selektive Prozesse, im Rahmen ökologisch relevanter Zeit-Skalen. Nahezu lethale Stress-Intensitäten resultierten hierbei in einer Populations-Morphologie, die deutlich von der einer weniger gestressten Population abwich.

Diese Studie konnte zeigen, dass Foraminiferen-Biometrie (trotz ihrer uni-zellulären Organisationsstufe) komplex auf Umweltänderungen reagiert. Die beobachteten Reaktionen werden vom Zusammenspiel der abiotischen Umwelt, biotischer Stress-Reaktionen und kryptischer Diversität beeinflusst, so dass weitere Studien notwendig sind um diese Probleme zu minimieren.

# Part I

# Synopsis of work

# 1 Introduction

## 1.1 Phenotypic plasticity, developmental stability, and the theory of shape

The shape of organisms has always been used to distinguish different 'kinds' of animals and plants, in the earliest days mostly for practical purposes. Later, scientists began to look closer into the topics of shape and form, and began to gradually develop a system by which the natural world could be divided into smaller units for descriptive purposes (e.g. Galilei 1638). The work by Linnæus (1758) established our current taxonomical system, which divides the biosphere into species, genera, and higher taxa based on morphology. This morphospecies-concept, which would distinguish species purely on the basis of their morphological characteristics, was for a long time the prevailing method for systematics, and is still often applied today.

Based on observations of the morphology of organisms, scientists began to realize that shape is not an invariant parameter within a taxon, but that it can vary as a result of exogenic forcing during the life of the organism in what is called phenotypic plasticity (e.g. Bumpus 1898). From those early beginnings, the field of morphometrics began to develop, which studies shape variation and its covariance with other parameters within taxa (Bookstein 1991). Until the early 1990s, morphometric approaches commonly applied multivariate analyses of traditional morphological parameters like length measurements (Jolicoeur 1963, Sundberg 1989). Thereafter, new concepts of morphometric analyses were developed, that could accommodate the analysis of shape as a whole, instead of a collection of individual measurements. Those new concepts were the outline analyses, in which the outer contour of an organism is described by mathematical shape descriptors derived from a large set of outline point coordinates, and geometric morphometrics, in which shape is described by the relationship of size-normalized landmarks towards each other (Adams et al. 2004). These new approaches, which were considered a revolution by Rohlf and Marcus (1993), inspired a much more detailed analysis of the shape of individual organisms, its relationship to external parameters, and its evolutionary value.

To better understand the nature and potential implications of phenotypic plasticity, research soon focused on developmental stability within individuals. In principal, the two aspects that influence phenotypic plasticity are variation and variability (compare Wagner and Altenberg 1996). Variation is the observed phenotypic plasticity of a community, which is the sum of the deviation of all individuals from the grand mean of the population. In contrast, variability describes the potential of the community to vary within the limits of its genetic constraints. The variability of a population, i.e. the range of possible phenotypes given the genetic background, is often much larger than the actually observed variation. The discrepancy between both, i.e. the fact that populations do not under all circumstances exhibit the full spectrum of their potential morphological variation, is in agreement with the concept of microenvironmental canalization (Waddington 1942, Schmalhauzen 1949) and stabilizing selection (Van Valen 1965). Microenvironmental canalization describes a process that yields a stable phenotype in a variable environment by buffering the phenotype against deviations, and is closely linked to stabilizing selection that reduces the variation in the community (Zelditch et al. 2012). Both concepts imply a selective process on the community that removes certain morphologies from the population by natural selection. As a consequence, a relationship between developmental stability (i.e. the ability of each individual to constrain its form during growth irrespective of environmental protrusions) and canalization within the population has been proposed (e.g. Debat and David 2001, Hallgrímsson et al. 2002).

Indeed, several subsequent studies found a relationship between developmental stability and individual fitness (Clarke 1993, Lens et al. 2002, Hendrickx et al. 2003, Beasley et al. 2013, De Coster et al. 2013, Sánchez-Chardi et al. 2013). Developmental stability would then provide a link between individual stress and a selective component in the population, perpetuating the idea of a universal relationship that would allow morphological parameters to be invoked as proxies for environmental stress. Nevertheless, this idea has not stood unchallenged for long. On the one hand, it was argued that while the environment could influence individual morphologies, the morphology would not necessarily impact the individual fitness. Morphology could thus not be coupled with canalization, because selection would work on other traits than the phenotype. If that would be true, then there should be no selective process that could alter the phenotypic composition of the population (Hoffmann and Woods 2001, Santos et al. 2005). On the other hand, the concept of biological integration (Klingenberg 2008) was invoked to argue against the impact of developmental stability on population morphology. To that end, some studies have shown

that several morphological parameters can be controlled by the same genes, so that those parameters are biologically integrated and could not vary independently of each other (Breuker et al. 2006, Breno et al. 2011, Klingenberg et al. 2012). This idea can be developed up to the level were canalization is hypothesized to be the natural development in any system that reached a certain amount of complexity and interlacement. This is because it was hypothesized that in such a system, virtually all involved parts are interdependent and no part can vary independently (Siegal and Bergman 2002).

Despite those uncertainties about the broad applicability of particular aspects of easy models, the mechanistics behind phenotypic plasticity are basically well understood (Klingenberg 2008). After Wagner (1996) and Wagner and Altenberg (1996) developed the theory of modularity, morphology was seen as a complex interaction between genes and phenotypic traits. Importantly, while the model suggests biological integration within so-called modules (i.e. phenotypic traits that are mainly controlled by the same genes) it allows broad independence between modules. Elaborating on that idea of modularity, Klingenberg (2003) introduced the model of developmental mapping (Fig 1.1). This model combines biological modularity with covariance between traits within modules due to interaction, non-linear genetical control of development pathways, and the modifying influence of external factors.

Following on those conceptual ideas, recent studies progressively tried to understand the link between variability and variation (e.g. Hoffmann and Woods 2001, Willmore et al. 2005, Debat and Peronnet 2013) and what factors control variation. To that end, the consolidation of chaperone proteins is widely believed to be the mechanistic base of controlling developmental stability during individual growth (e.g. Smith et al. 2005, Debat et al. 2006, Takahashi et al. 2010, Takahashi et al. 2011). This hypothesis suggests, that production of such proteins could stabilize the morphology of an organism during growth, reducing developmental instability. This is achieved by the chaperones mediating the correct folding of vital proteins in the cell that would otherwise become denaturated due to the effects of a stressor acting on the organism. While this mechanism might be further complicated by high degrees of interactions between genes (Breuker et al. 2006, Hayden et al. 2012) or genes and the environment (Milton et al. 2003, Hannig 2013) it seems to overall offer a valuable explanatory basis for further research.

On an individual basis, developmental stability is often quantified using the asymmetry of an organism. For this purpose, three types of asymmetry can be

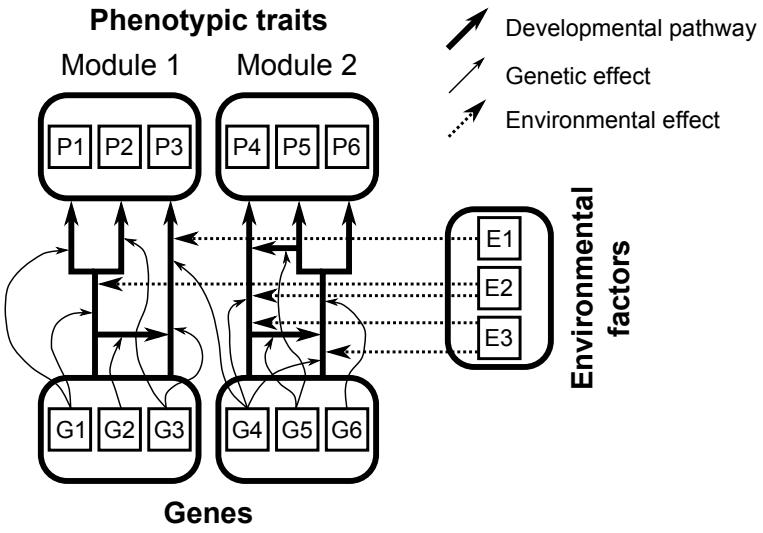



**Figure 1.1** Scheme of the theory of developmental mapping (Klingenberg 2003). Instead of being directly influenced by the genes, phenotypic traits are developed via developmental pathways predetermined and on various occasions influenced by one or several genes. These pathways can interact with each other, so that not all traits within the same module can vary independently and are biologically integrated. Different modules are widely independent of each other, but occasionally the same genes control phenotypic traits in different modules. Environmental factors can further modify developmental pathways within the borders of variability, leading to phenotypic plasticity. Redrawn and modified after Klingenberg (2008, fig. 4).

distinguished (compare Graham et al. 1993): (1) directional asymmetry, (2) antisymmetry, and (3) fluctuating asymmetry. Directional asymmetry results from a one-sided or directional trait on the population level being predominantly developed on one side of the organisms symmetry axis or into one direction, as for instance the heart of mammals (which is predominantly developed on the left side if the bilateral axis). Antisymmetry means that a one-sided trait is equally often found on either side of the organisms symmetry axis or into both directions within the population. Due to this definition, the same general trait can be displaying either directional asymmetry or antisymmetry, depending on the species in question. Most planktonic Foraminifera, for instance, show no preferred coiling direction—and coiling direction therefore would be antisymmetric in those species—while some species (e.g. *Globigerinoides sacculifer*) prefer one coiling direction, so that this trait shows directional asymmetry in that species (Brummer and Kroon 1988). Fluctuating asymmetry refers to an instability in morphology induced by the inability to retain a symmetric growth pattern during ontogeny. This concept in particular was originally designed for bilateral organisms, but can be adapted to planktonic Foraminifera by

investigating their chamber-by-chamber growth pattern (compare Manuscript 3).

### 1.1.1 Selective mechanisms and patterns in morphology within a population

To leave a discernible imprint within a population, certain trends in individual morphology have to accumulate within the population over time. Such manifestations of developmental patterns within a population, may they be morphological or otherwise, are the consequence of selective patterns that favour certain traits over others, leading to changes in the distribution of traits over considerably long time intervals. The two main patterns of selection in this regard that alter the perceived variation in a population are either stabilizing selection or disruptive selection.

Stabilizing selection (Schmalhauzen 1949, Van Valen 1965) leads to a narrowing of the reaction norm (i.e. the width of the phenotype distribution curve) of a population by favouring a mean phenotype over extremal phenotypes in individuals. Stabilizing selection is often associated with stable environments, but has also been shown to be resulting from fluctuating selection in a highly unstable environment, where a mean trait is only of statistical advantage (Pélabon et al. 2010). The concept of stabilizing selection channels into the idea of canalization (Waddington 1942, Schmalhauzen 1949, Wagner et al. 1997), which itself can be separated into environmental canalization (e.g. Debat and David 2001, Hallgrímsson et al. 2002, Willmore et al. 2005) and genetic canalization (e.g. Kawecki 2000). Environmental canalization is easily perceived as the reduction of phenotypic plasticity in regard to environmental influences. It is therefore closely linked to developmental stability, i.e. the ability of each individual to constrain its growth pattern irrespective of environmental forcing (Debat and David 2001, Klingenberg 2003). Genetic canalization, on the other hand, is a reduction in variability of the community by selecting against genes that would theoretically allow for higher variation. Neither the relationship between environmental and genetic canalization (Hansen 2006), nor the exact mode of selection that could influence the variability instead of the manifestation of a trait are yet well understood, however (compare Pélabon et al. 2010, and references therein).

Disruptive selection, in contrast, is the broadening of the reaction norm of the population (Schmalhauzen 1949, Bull 1987), which often but not always leads to a bimodal distribution of traits (Doebeli 1996). It is therefore often associated with decanalization when active over long time scales, although decanalization has also been found to result from directional selection (Hayden et al. 2012). Such decanal-

ization can result in bet-hedging (Slatkin 1974, Philippi and Seger 1989), which is a process that can serve to enhance survivability of a species under unfavourable environmental conditions. The fitness of the population equals the geometric (in contrast to arithmetic) mean of the fitness of all individuals. Therefore, a lower but rather constant fitness of all individuals can result in a higher population mean fitness than a bimodal fitness distribution with some individuals having much higher fitness while others have very low fitness. The bet-hedging hypothesis therefore predicts that under unfavourable, especially variable environmental conditions it can be a selective advantage to have offspring that has a high variation. In this way, chances are maximized that at least part of the offspring will be adapted to future environmental conditions, resulting in a stable, mediocre fitness of all individuals.

Directional selection, in contrast to the two other modes of selection described before, changes the mode of a population trait from one status to another. By itself, directional selection does not change the width of the reaction norm, but it can often lead to decanalization with a result similar to disruptive selection 'when genotypes with new phenotypes sweep through a population' (Hayden et al. 2012, p. 1).

Over long enough time intervals, those modes of selection are inherently responsible for evolutionary patterns, with the variability of a population directly influencing its evolvability (i.e. its potential to evolve, Wagner and Altenberg 1996). While it was commonly believed that evolution would mainly result from a directional change, associated with a clear trend in the population mean trait, it recently transpired that evolution might more often act on a random walk pattern (Hunt 2007, Bookstein 2013). This means, that directional selection may not play as important a role in evolutionary processes, but that other (more random) means of selection are equally important to explain evolutionary patterns.

In conclusion, while some of the mechanistics behind phenotypic plasticity are well understood, there is still a need to establish how environmental stress impacts the morphology of a population over time. Especially, it needs to be established to what degree the morphology truly represents the amount of environmental stress a population was exposed to, and how morphological reactions are linked to environmental forcing.

To study these questions, it is important to use a representative model system. Such a model system must allow to track the morphological reactions of a population to environmental stress/change over ecologically effective timescales. Since such timescales cannot be simulated in the laboratory, but must rather be based on natural experiments, a suitable model system must allow to quantify morphological change

by providing high individual numbers of specimens which allow to draw reliable conclusions. Calcareous marine microplankton, such as planktonic Foraminifera, occur in large abundances and show a high fossilisation potential (Kučera 2007). They thus provide a representative model system to address those questions, and have therefore been chosen for the studies presented in this thesis.

## 1.2 Introduction to planktonic Foraminifera

The taxon Foraminifera comprises a taxonomically diverse group of unicellular eukaryotes, many of which are characterized by the possession of a biomineralized shell[1]. Their phylogenetic position was uncertain for a long time, and only relatively recent molecular analyses have revealed their evolutionary relationships with other protist taxa (Caron et al. 2012). Foraminifera now belong to the taxon Rhizaria (compare Keeling et al. 2009), within which they form a monophyletic group branching within the now paraphyletic 'Radiolaria' (Sierra et al. 2013). As is typical for the taxon Rhizaria, the cytoplasm of Foraminifera forms characteristic flow-structures called rhizopodia (Allen 1964). The taxon can be separated into benthic and planktonic Foraminifera, which is an artificial subdivision purely on the basis of their lifestyle. Accordingly, most Foraminifera species have a benthic lifestyle, but (probably repeated) invasions of the open water column occurred, leading to a low diverse but numerically large community of planktonic Foraminifera. The latter group is the focus of this thesis. While benthic Foraminifera comprise a taxonomically highly diverse group, which originated at least in the Cambrian and is rather variable concerning their shell mineralogy and ultrastructure (compare Armstrong and Brasier 2005, and references therein), planktonic Foraminifera are a lot more homogeneous in both regards.

Planktonic Foraminifera are exclusively marine, and first evidence for an invasion of the plankton dates to the Early to Middle Jurassic (Hart et al. 2002). Currently, the taxon contains 47 extant morphospecies belonging to 21 genera (compare Hemleben et al. 1989, Darling et al. 2006, Darling et al. 2009, Aurahs et al. 2011, Weiner 2014, Weiner et al. 2015). It is likely that recent planktonic Foraminifera are a polyphyletic group, which were derived independently from at least two ancestral lineages of benthic Foraminifera (Darling et al. 1997). Nevertheless, they all share a comparable shell ultrastructure and morphology. The shells of planktonic Foraminifera are

[1] Traditionally, the shell of Foraminifera is also called 'test'.

composed of calcite, and consist of several chambers that are built in consecutive order throughout ontogeny. Foraminifera construct new chambers throughout their entire lifespan, which equals several weeks to a few months in most species but possibly considerably longer in others (Bé 1977). The shells are perforated by pores and normally have one or more larger openings which are called apertures. Through these openings, rhizopodia can protrude outside the shell to interact with the environment and facilitate the intake of nutrients, while oxygen uptake is mainly conducted via the pores (Bé 1977, Hemleben et al. 1989, Schiebel and Hemleben 2005). Upon building a new chamber, the old aperture becomes a foramen, which shell-internally connects older chambers with each other (Hemleben and Bijma 1994, Schiebel and Hemleben 2005). During the formation of new chambers, the rhizopodia construct a radial cytoplasm network that is then surrounded by a primary organic membrane which in a consecutive step is calcified to form a new chamber (Bé et al. 1979) (Fig. 1.2). Several species posses spines on their shell surface, which are elongated, flexible structures composed of calcite that radially protrude from the shell.

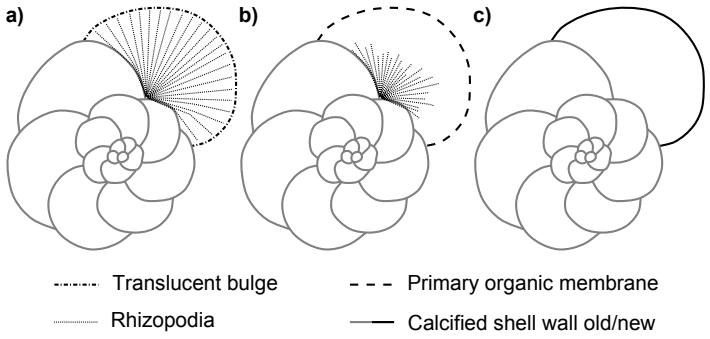



**Figure 1.2** Chamber formation: Radially streaming rhizopodia form a translucent bulge (a) where a primary organic membrane is secreted (b) and then calcified (c). Redrawn and modified after Grell (1973) and (Bé et al. 1979).

On the basis of different features of the shell structure, four different morphogroups of planktonic Foraminifera can be distinguished, as is shown in Fig. 1.3 (compare also Hemleben et al. 1989). In the microperforate group (Fig. 1.3a, f), pores are considerably smaller than 1 µm in diameter, and therefore not visible in light microscopic (LM) observation, where the surface rather appears soft and velvet-like. In scanning electron microscope (SEM) observations it is evident, that the shell is perforated by numerous small pores and covered with little pustules, but is lacking spines.

The other three morphogroups have pores with diameters $> 1$ µm and are thus summarized within the macroperforate group, but can be distinguished on the basis of presence or absence of spines and shell structure. The spinose (Fig. 1.3d, i) and

non-spinose (Fig. 1.3c, h) Foraminifera morphogroups both have a bilamellar shell, where a calcitic layer is secreted on both sides (internal and external) of the primary organic membrane (Fig. 1.3e). The spinose morphogroup possesses calcitic spines which reach down to the primary organic membrane, are round in cross-section, and mainly serve as a floating and prey capture device for the cell. Instead of spines, pustules are covering the shell surface of the non-spinose morphogroup.

Species of the monolamellar group (Fig. 1.3b, g), as the fourth main morphogroup of planktonic Foraminifera, do posses both spines and large pores, but are set apart from the spinose group by their monolamellar shell structure. In this morphogroup, the shell wall is reinforced by a calcitic layer that only covers the exterior side of the primary organic membrane, and it is covered with thick but fragile spines, which are only attached to the shell surface and are triangular in cross-section (Fig. 1.3e). Phylogenetically all four morphogroups of planktonic Foraminifera seem to be monophyletic (Pawlowski 2000, Aurahs et al. 2009).

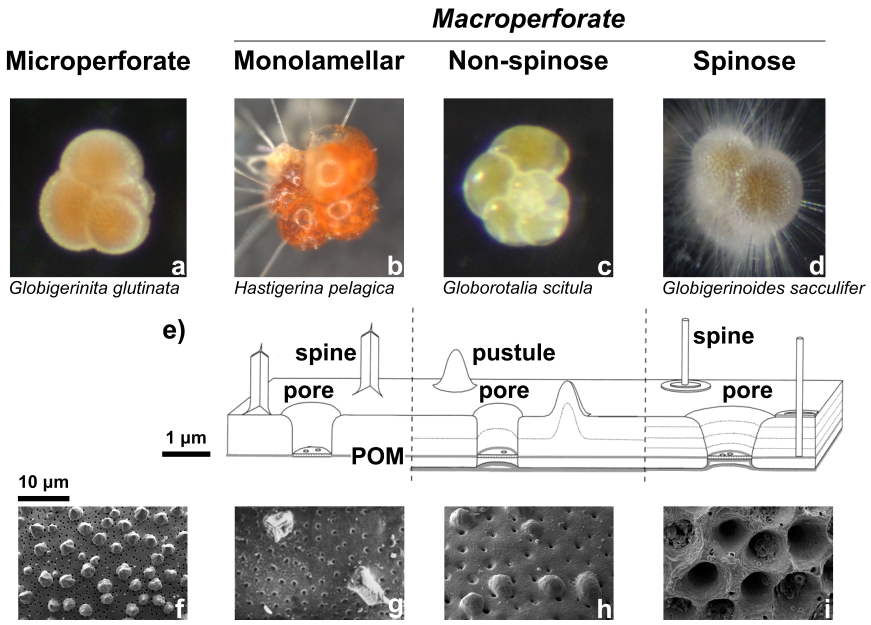



**Figure 1.3** Planktonic Foraminifera are commonly divided into four different morphogroups, based on their shell structure, as shown here by light microscopic (LM) images of exemplary species (a–d), schematics of the shell wall (e), and scanning electron microscope (SEM) close ups (f–i). The primary organic membrane (POM) seals the pores and serves as calcification seed during chamber formation (e, compare Fig. 1.2). Modified after Schiebel and Hemleben (2005, fig. 1) and Kučera (2007, fig 2). Additional image sources: *G. glutinata*, *G. scitula*, and *G. sacculifer* LM images – Workgroup Mikropalaeontology–Palaeoceanography (2013); *H. pelagica* LM image – WG Micropalaeontology–Palaeoceanography; *H. pelagica* SEM close up – B. Hayward, WoRMS, `http://www.marinespecies.org/photogallery.php?album=772&pic=38545`

The abundance and diversity of planktonic Foraminifera is regionally and locally highly variable. Abundances reach from $> 1000\,\text{specimens}\,\text{m}^{-3}$ in polar regions during bloom conditions to $< 100\,\text{specimens}\,\text{m}^{-3}$ in oligotrophic waters (Schiebel and Hemleben 2005). The diversity follows a standard pattern, with low diversity and high individual abundances in the polar regions and increasing diversity towards the equator (Rutherford et al. 1999, Kučera et al. 2005). Despite the fact that only *c.*25 per cent of the carbonate primarily produced by planktonic Foraminifera settles to the ocean floor, they are important marine calcifiers, which contribute between 30 and 80 per cent to the total carbonate budget that is deposited above the calcite compensation depth in the world oceans (Schiebel 2002).

Planktonic Foraminifera are primarily heterotrophic (Schiebel and Hemleben 2005). The foraminifer is floating in the water, and food particles that come in contact with the rhizopodia are transported towards the shell and digested in food vacuoles within the cell. Non-spinose Foraminifera are largely believed to be herbivorous and/or suspension feeders, but species of the spinose and monolamellar morphogroup are normally carnivorous (Schiebel and Hemleben 2005). The spines help to significantly increase the cross-sectional area of the individual, promoting the chance of prey to be captured. When a prey organism touches the spines it is immobilized by the cytoplasm that flows around them and also by the flexible spines which bend around the prey, forming a weir-like structure. The foraminifer then proceeds by dissecting the prey via cytoplasm activity and transporting bits of it towards the shell for digestion (Anderson and Bé 1976). Planktonic Foraminifera are rather flexible concerning their prey size, which can be much larger than the foraminifer itself, and are not limited to unicelullar prey organisms—conversely, metazoans like copepods and nauplius larvae are frequently consumed by larger Foraminifera like *Hastigerina pelagica* (Anderson and Bé 1976). While all planktonic Foraminifera are heterotrophic, some species engage in symbiosis with algae, mainly dinoflagellates (Bé and Hutson 1977, Gast and Caron 1996) and occasionally other taxa like haptophytes (Gast et al. 2000). The symbionts are not inherited from the mother cell, but are gathered from the open water during the early life of the foraminifer (Hemleben et al. 1989).

Apart from symbiont-bearing species, which are restricted to the photic zone of the upper water column due to light requirements (Bé and Hutson 1977, Bé et al. 1982), planktonic Foraminifera as a group inhabit a wide depth-habitat. Within the taxon, however, different species seem to be more or less restricted to, or at least most abundant in, species-specific depth-ranges (Pujol and Vergnaud Grazzini 1995, Kuroyanagi and Kawahata 2004, Schiebel and Hemleben 2005). It has been

suggested, however, that the preferred depth habitat of species might change during ontogeny, resulting from a postulated vertical migration during their life-cycle (Emiliani 1971, Schiebel and Hemleben 2005). Such a migration pattern could be enforced by the fact that in contrast to benthic Foraminifera, sexual reproduction by fusion of two gametes into one zygote is currently the only known mode of reproduction in planktonic Foraminifera (Hemleben et al. 1989). Resulting from their low abundance in the world oceans, it is perceivably difficult to ensure the encounter between two gametes which, despite their ability for active locomotion using two flagella, must be very limited in their range due to energy constraints of their small size (Hemleben et al. 1989). Accounting for this problem, both vertical migration into a very limited depth interval (Emiliani 1971, Schiebel and Hemleben 2005) and temporal synchronization of reproduction (Spindler et al. 1979) have been invoked to solve this problem by spatially and temporally maximizing the gamete density to significantly increase chances for encounter. The empty shell of the mother cell settles down into the sediment as soon as the gametes have been released. While individual Foraminifera could potentially die by other causes prematurely, it is believed that the majority of shells found in the sediment (and sediment traps, for that matter) are from adult specimens that underwent gametogenesis.

It is remarkable that the size range of planktonic Foraminifera spans a whole order of magnitude, even when only regarding adult specimens. The taxon includes species like *Berggrenia pumilio* or *Turborotalita clarkei*, which shells are often not much more than 50 µm in diameter, and *Hastigerina digitata* or *Orbulina universa*, which can reach final sizes of approximately 1 mm (compare Hemleben et al. 1989).

A persistent pattern in planktonic Foraminifera that has been revealed during the last decades is their commonly high degree of cryptic speciation. Early approaches of molecular analyses hinged towards the existence of a higher biodiversity of the group than was commonly believed at the time and could be deduced from morphology alone (e.g. Darling et al. 1997, de Vargas et al. 1999). Subsequent analyses revealed that many commonly acknowledged morphospecies[2] within planktonic Foraminifera actually comprise a few or even several different biospecies[3] (compare Darling and Wade 2008), which are partly characterized by differing environmental requirements (e.g. Darling et al. 2003, Weiner et al. 2012). While more detailed investigations have shown that some genetic types can indeed be differentiated on the basis of morphological characteristics that were formerly believed to represent

[2]Organisms with a similar morphology are combined into one species.
[3]Organisms which form a reproductive community are combined into one species.

phenotypic plasticity (Morard et al. 2009, Aurahs et al. 2011, Morard et al. 2011), other morphospecies still exhibit an unconstrained degree of cryptic diversity (e.g. Huber et al. 1997, Weiner et al. 2015). Contrasting to this general pattern of cryptic or pseudo-cryptic (i.e. species are only distinguishable by minor morphological differences) speciation, the reverse can also exist. For instance, in *Globigerinoides sacculifer* it was found that despite the spectrum of morphological plasticity within the plexus, which resulted in the traditional subdivision into several species, the whole morphospace is accommodated within a single biological species (André et al. 2013). Morphology and biological systematics in planktonic Foraminifera therefore seem not to be uniformly related to each other.

### 1.2.1 Biometric analyses of planktonic Foraminifera

Biometrics is an expansion of morphometrics, that not only covers morphology s.str. but also other aspects of phenotypical traits like shell calcification. The biology of planktonic Foraminifera makes this group of marine protists ideally suited for biometric analyses that would be impossible in many other protist groups. Their shell mineralogy, which is not susceptible to dissolution, together with their relatively high abundance in the world oceans means that they comprise a rich and representative record (Kučera 2007). As such, they are readily available in statistically useful abundances in sedimentary material and recent samples taken with plankton nets or sediment traps alike. Furthermore, due to their unique shell structure, the whole ontogenetic growth history is preserved within the shells, allowing to reconstruct the whole lifespan of each individual cell. While cryptic diversity can be a problem in such analyses (compare for instance Naik et al. 2013, Thirumalai et al. 2014), using current knowledge about minor morphological differences and/or distribution patterns of (pseudo-)cryptic species can widely circumvent such problems. Conversely, in species where such cryptic diversity can be precluded, often a high degree of morphological disparity can be observed (Caron et al. 1982, Caron et al. 1987, André et al. 2013), which presumably must then be driven by exogenic factors. In contrast to what might be expected, relatively few studies to date approached the problem of the use of biometric analyses in planktonic Foraminifera for reconstructing past environmental conditions or environmental stress levels. In part, this is likely due to the fact that environmental research in planktonic Foraminifera mainly focuses on shell geochemistry and transfer functions (e.g. Erez and Honjo 1981, Spero and Lea 1993, van Eijden and Ganssen 1995, Niebler et al. 1999, Kučera et al. 2005, Siccha

et al. 2009, Lin et al. 2011), while widely neglecting shell biometry.

Most studies investigating shell morphology focused on the incidence of clearly abnormal growth forms (i.e. chamber insertions, torsions, and shell excesses) in benthic Foraminifera (Alve 1995, Geslin et al. 2000, Geslin et al. 2002, Ballent and Carignano 2008, Debenay et al. 2009). Several possible explanations for those irregularities have been proposed, including anthropogenic pollution, salinity changes, and shell regeneration (compare Boltovskoy et al. 1991, Geslin et al. 2000). Such studies are problematic, because they are dependent on what one considers to be 'abnormal' and also suffer from the large uncertainty associated with the commonly low abundances of abnormal morphotypes. Furthermore, it is not always easy to constrain the environmental factor that was responsible for the morphological reaction. While some authors propagate the applicability of such analyses for environmental pollution-monitoring (Alve 1995, Le Cadre and Debenay 2006, Debenay et al. 2009), others object that environmental stress due to natural changes like sediment input can cause comparable signals (Geslin et al. 2002).

Within planktonic Foraminifera, biometric analyses to a large degree have been focused on shell size and shell calcification. Shell size is often hypothesized to be linked to optimal growth conditions (i.e. the sum of all environmental factors is close to the optimum of the species), with a larger mean size of the population indicating more suitable environmental conditions (Hecht 1976, Malmgren and Kennett 1978b). This phenotypic trait was subject to some research over the years (Schmidt et al. 2006), and the hypothesis of a correlation between shell size and optimal growth conditions has been tested independently by approximating optimal environmental conditions on the basis of the temperature preferences of individual species (Schmidt et al. 2004). Other studies, however, found more straightforward explanations. Haenel (1987), for instance, found that shell size in *Orbulina universa* is linked to sea water salinity (seemingly undisturbed by environmental stress levels), which he interpreted as an adaptation to obtain stable buoyancy in water of different densities.

Shell calcification in planktonic Foraminifera has been an emerging field of study from the late 1990s onwards. Starting as a by-product of understanding isotope incorporation into the shells (Spero et al. 1997, Bijma et al. 1999) it soon emerged as a research topic in its own right (e.g. Broecker and Clark 2001, Barker and Elderfield 2002). The idea, inspired by Lohmann (1995), was, that shell calcification in planktonic Foraminifera should be mainly influenced by the carbonate saturation state of the sea water. Should this be true, calcification intensities (i.e. the amount of

calcite present in the adult, post-gametogenetic shell) of foraminiferal shells could be a versatile proxy for past and present saturation states and acidification values of the oceans. This proxy could in theory be complicated by the presence of gametogenetic calcite, which is a calcite layer deposited on the shell surface in preparation of gametogenesis, and thus not part of the ontogenetic shell calcification (Bé 1977, Hemleben et al. 1989). However, recent studies suggest that the contribution of this gametogenetic calcite has been drastically overestimated in earlier studies (Hamilton et al. 2008), and it might therefore not pose a serious problem in that regard. It was assumed that shell calcification is strongly influenced by carbonate saturation of the ambient sea water, but that other factors like temperature and productivity also play an influencing or at least mediating role (for details compare Manuscript 2, especially Table 5.6). Further, relationships between shell calcification intensity and environmental factors seem to be species-specific (Table 5.6), and to a degree also biotically controlled by environmental suitability, widely disentangled from direct influence by one particular environmental factor (de Villiers 2004, Naik et al. 2013).

More advanced morphometric analyses have only been applied on Foraminifera on rare occasions, and if so with varying results. A number of studies have found links between certain—seemingly narrowly restricted—morphological parameters (e.g. lobateness, porosity) and particular environmental factors like salinity or temperature (Malmgren and Kennett 1976, Baumfalk et al. 1987). Interestingly, some of these studies suggest that those morphological parameters are exclusively influenced by the environmental factor itself, without further modification by the environmental stress reaction which results from changes in that parameter (Malmgren 1984, Baumfalk et al. 1987). Yet other studies, while further elaborating shell size as being influenced by environmental factors, did not find any clear results in other morphological parameters at all (Moller et al. 2013). However, due to the relatively small effort yet invested in this particular research topic, there are not enough data for any conclusive interpretations of the influence of the environment on foraminiferal shell morphology.

## 1.3 Motivation and objectives of this work

Planktonic Foraminifera are already widely used as environmental proxies in geochemical and transfer function analyses (Guiot and de Vernal 2007, Ravelo and Hillaire-Marcel 2007). They have proven to be a useful model system in both regards, owing to their excellent fossilisation potential and relatively high abundance in the

marine plankton that ensures large sample sizes (Kučera 2007). Biometric analyses, on the other hand, have been applied relatively scarcely to the foraminiferal record.

Planktonic Foraminifera are ideal candidates for biometric analyses within the microplankton, because their unique shell growth structure records the entire life cycle of an individual (Fig. 1.2). This makes it possible to reconstruct influences that occurred during the ontogenetic development of the specimen. Therefore, it is worthwhile to enhance our understanding of foraminiferal morphology, to make it available as an environmental proxy. Especially so, when keeping in mind that the traditional environmental proxies are also subject to partly uncontrollable error terms (Ganssen et al. 2011, Telford and Kučera 2013). Additionally, since natural selection mostly works on the phenotype, understanding phenotypic changes in response to the environment can increase our understanding of evolution within the taxon.

While the concept of morphological malformation in response to environmental stress is relatively well understood (Debat et al. 2006, Takahashi et al. 2011) and is already widely used in phylogenetically more advanced taxa (e.g. Clarke 1993, Leung et al. 2000, Hendrickx et al. 2003, Beasley et al. 2013, De Coster et al. 2013, Sánchez-Chardi et al. 2013), its role in foraminiferal shell formation remains to be established. Several studies suggest that extreme environmental perturbations, like near-lethal poisoning, can lead to severe malformations in foraminiferal shells (e.g. Alve 1991, Sharifi et al. 1991, Coccioni 2000, Burone et al. 2006, Le Cadre and Debenay 2006, Frontalini et al. 2009), and that comparable malformations are found in the fossil record (Ballent and Carignano 2008). However, only very few studies (e.g. Malmgren and Kennett 1976, Malmgren 1984, Moller et al. 2013) have looked into a more thorough analysis of subtle morphological deviations of the chamber-by-chamber growth pattern of Foraminifera. While extremely abnormal shells are immediately obvious, they only occur under extreme conditions that are rarely found in nature, and are often fatal to the community. Deviations in the small-scale shell morphology, on the other hand, are not easily detectable, but bear the potential to occur in varying intensities and manifestations across a wide range of environmental conditions. Understanding their pattern thus offers the possibility to use the overall shell morphology in Foraminifera as a versatile environmental proxy that can be indicative of a variety of environmental changes and environmental stress.

Shell calcification of planktonic Foraminifera is principally the only already widely applied biometric trait, that has been used to reconstruct parameters of the oceanic water column. Nevertheless, this proxy still has some limitations. There is wide

disagreement about the nature of environmental influence on foraminiferal shell calcification. The ideas of which parameter exactly influences shell calcification in planktonic Foraminifera range from oceanic $CO_3^{2-}$ contents (e.g. Lohmann 1995, Barker and Elderfield 2002, de Moel et al. 2009, Marshall et al. 2013) over sea surface temperature (Manno et al. 2012, Davis et al. 2013) and phosphate content of the sea water (Aldridge et al. 2012) to a pure biological factor as function of overall environmental suitability, that is not influenced by any particular environmental parameter (de Villiers 2004). Further experiments are therefore needed to solve some of those issues, so that shell calcification can be adopted as a reliable environmental proxy.

All biometric analyses further suffer from the problem of cryptic speciation within planktonic Foraminifera. Cryptic species have been shown to occasionally favour different environments (e.g. Darling et al. 2003) and pseudo-cryptic species differ in minute morphological characteristics (e.g. Renaud and Schmidt 2003). Arguably, a biometric deviation observed in any population could therefore also be the result of changing abundances of several unperceived pseudo-cryptic species in the population. Likewise, some candidate morphologies amongst planktonic Foraminifera exist which where conceived to be ecophenotypes of the same species, while in reality they are different biospecies. Traditionally, those species have been combined for analyses (e.g. Wang 2000) and it is therefore of great importance to understand how this artificial pooling disturbs observed patterns.

In order to investigate the impact of environmental change and stress on the biometry of planktonic Foraminifera the null-hypotheses of the present thesis were defined as follows:

1. Shell calcification intensity in planktonic Foraminifera is homogeneously influenced by one environmental parameter, and there is no difference in reaction between different species!

2. Shell morphology in planktonic Foraminifera does not show any deviations in regard to environmental stress, but is rather robust against environmental fluctuations!

If Hypothesis 1 would be correct, then this would imply that shell calcification intensity in planktonic Foraminifera is an unproblematic proxy for exactly one environmental parameter, completely decoupled from the actual species used for an analysis. If Hypothesis 1 could be disproven, however, the application of this biometric parameter would be more complicated. Shell calcification intensity could still

be a versatile proxy to reconstruct past environments, but only if certain precautions would be taken. First, if shell calcification intensity is influenced by more than one environmental parameter, it could not be applied to reconstruct past environments straightforward. Rather, it could only be used as a proxy for one environmental parameter, if fluctuations in other influencing parameters could be either excluded or independently quantified. Second, if shell calcification intensity and its relationship to one or more environmental parameters is not fully comparable between species, then this means that only species-specific analyses are comparable with each other. Importantly, this would imply an unreliability of analyses for which it cannot be excluded that several pseudo-cryptic species were pooled together in the analysis.

If Hypothesis 2 would be true, then shell morphology of planktonic Foraminifera could not be used as an environmental proxy of any kind. If this hypothesis could be disproven, then shell morphology could be a versatile proxy to reconstruct past environments. It would then be necessary, however, to test this proxy for its dependence on changes in particular environmental factors as well as its dependence on the species. In this case, the derived Hypothesis 2.1 could be formulated as 'Shell morphology in planktonic Foraminifera is homogeneously influenced by one environmental parameter, and there is no difference in reaction between different species'. The falsification of this hypothesis would have equivalent implications to the falsification of Hypothesis 1 for shell morphology instead of calcification intensity.

# 2 Material and methods

## 2.1 Choice of sampling material

Investigating the proposed research questions required sample material that fulfilled certain requirements. First, the sample material had to the furthest possible extent limit the problem of cryptic speciation, so that observed trends could be safely interpreted as morphological changes instead of species-composition changes. Second, the material had to be chosen such that observed trends are likely the reaction of a local community to environmental forcing, instead of the result of migration of another population with different morphology into the sampling area. For both aspects, it is beneficial to use material from relatively isolated marginal seas. Their limited water exchange with the open ocean limits migration of other populations and cryptic species into the area. To further constrain the problem of cryptic speciation, it is additionally beneficial to use samples from areas were the cryptic diversity of the investigated species is supposed to be low. Third, using settling foraminiferal shells instead of life specimens ensures that most specimens investigated are adults, and the observed trends are not likely to be complicated by ontogenetic patterns. Fourth, it had to be made sure that the populations were exposed to natural ranges of environmental change on ecologically effective timescales. Such timescales could not have been reproduced in the laboratory, but the sedimentary record and trap series are ideally suited for that purpose. Fifth, to investigate the effect of environmental change and stress on shell biometry, samples had naturally to be from environments that were subject to changes during the investigated time interval. In the ideal case, the environmental forcing was limited to a few factors, while others remained constant, so that influences of individual factors could be quantified.

In accordance with the above requirements, three sampling locations have been chosen to allow the assessment of planktonic Foraminifer biometrics under varying environmental parameters and stress conditions, including material from sediment cores and sediment trap samples. The environmental setting of the studied samples will be shortly summarized here, for a more detailed discussion the reader is referred

to the respective manuscripts included in Part II of this thesis. The position of all sampling localities is presented in Fig. 2.1.

Sedimentary material was taken from either gravity core M51-3/SL104 or piston core M5-2/KL09 (aka: Geo-TÜ/KL09). The core M51-3/SL104 was taken during the RV Meteor cruise M51 (Hemleben et al. 2003) in the Eastern Mediterranean, and the studied interval (128.2–124.4 ka) covers the Eemian insolation maximum, including the onset of the Mediterranean Sapropel S5 (Rohling et al. 2002) (Manuscripts 1 and 3). This sapropel occurred during the Eemian as a result of a failing vertical circulation of the water masses in the Eastern Mediterranean (Rossignol-Strick et al. 1982, Rohling et al. 2000), induced by increasing freshwater influx (Williams et al. 1978). This process resulted in a complex change of environmental parameters in the Eastern Mediterranean, including dysoxic deep-water layers and reduced salinity in the upper water column. Resulting from this development, local extinctions of several planktonic Foraminifera species can be observed in the entire Eastern Mediterranean at that time (Cane et al. 2002).

Piston core M5-2/KL09 was taken in the Red Sea during RV Meteor cruise M5 (Nellen et al. 1996), and the interval studied in the present work covers the time from 479.7 to 463.1 ka, including the onset of the aplanktonic zone associated with Marine Isotope Stage 12 (MIS 12) (Fenton et al. 2000) (Manuscript 4). Several aplanktonic zones occurred in the Red Sea during the Pleistocene, induced by glacio-eustatic sea-level changes and resulting increases in sea water salinity (Fenton et al. 2000). Aplanktonic zones are therefore barren of virtually any planktonic life, replicating the local extinction setup investigated in core M51-3/SL104. But in contrast to the Mediterranean core, the environmental stressors leading to local extinction in core M5-2/KL09 can nearly exclusively be limited to salinity increase.

Sediment trap material was taken from the JGOFS trap 53 at station L1/K276 in the northeastern Atlantic Ocean, close to the Azores Front (Manuscript 2). At this station, a time series of settling planktonic material was sampled between February 2002 and April 2003, using a sediment trap as described by Kremling et al. (1996). This time series spans one full year, and thus covers a full seasonality cycle at that station. This region is characterized by very low variabilities of the local carbonate saturation state and a high seasonality of temperature, salinity, and productivity (Ingleby and Huddleston 2007, `http://climexp.knmi.nl/`).

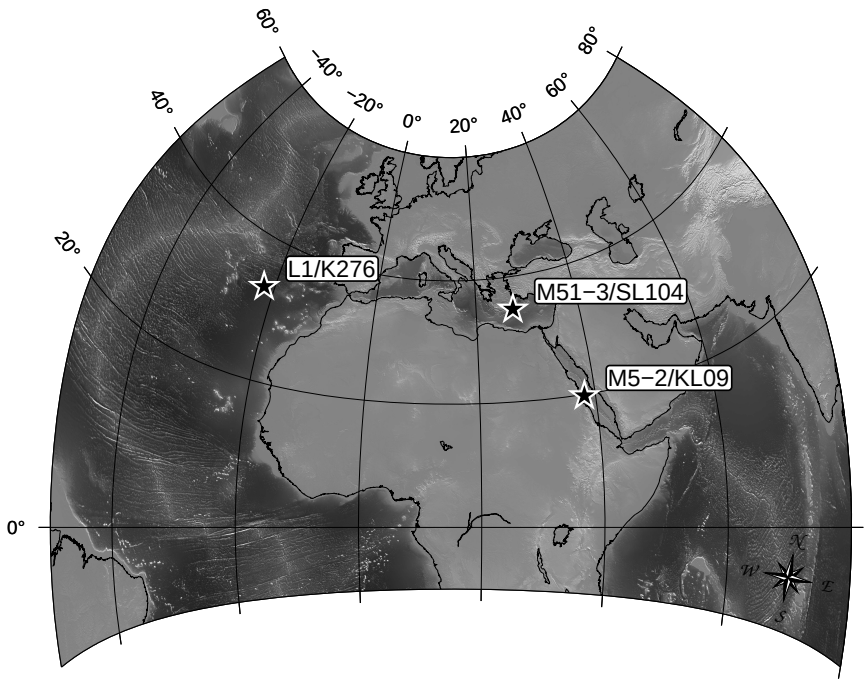



**Figure 2.1** In the present thesis, material from three different sampling sites was used. Manuscripts 1 and 3 are based on material from gravity core M51-3/SL104 (34.81° N, 27.28° E) taken in the Pliny Trench southeast of Crete in the Eastern Mediterranean (Hemleben et al. 2003). For Manuscript 2, material from the JGOFS sediment trap 53 at station L1/K276 (*c.*33° N, *c.*22° W) in the North Atlantic close to the Azores Front was used, which was collected during spring 2002 to spring 2003. Manuscript 4 is based on material from piston core M5-2/KL09 (19.80° N, 38.10° E) from the central Red Sea (Nellen et al. 1996). Topography based on ETOPO1 data (Amante and Eakins 2009).

## 2.2 Sample preparation

Material from sediment cores was sampled using a U-channel, i.e. a 'U'-shaped plastic-rail of 1 m length and roughly 2 cm width. For that, the core was cut in half and the U-channel was inserted lengthwise into the sediment material of the working-half of the core. The U-channel was then sealed at the bottom with a similarly shaped counter-piece as to remove a cuboid-shaped core sample. The core sample in the U-channel could then be cut into slices using a knife, and the thus derived samples were freeze-dried and stored for later use. For further processing, those samples were soaked in tap water and then sieved under flowing tap water over a 63 µm screen, with the fraction $< 63$ µm having been disposed. The fraction larger 63 µm was dry sieved into the fractions $< 150$ µm and $> 150$ µm, and the two fractions were stored separately in glass vessels. For the present studies the

fraction $> 150\,\mu m$ was used exclusively in order to only use adult specimens and thus eliminate the influence of ontogenetic processes.

In order to obtain sediment trap samples, a sediment trap containing a series of sampling cups that are replaced via rotation in a barrel on a pre-defined schedule, were fixed on the sea-floor with an anchor-like mooring. Sampling-cups of the sediment trap had been filled with a solution of four parts *in situ* sea water and one part 5 % sodium acide in order to inhibit biological activity and thus degradation in the sample cups during sampling (Storz et al. 2009). After recovery of the sediment trap, the sample cups were stored in the dark at approximately 4–6 °C for later analysis and freeze-drying. For analysis of planktonic Foraminifera, the freeze-dried sample material was soaked in distilled water and wet-sieved under flowing tap water over a 63 µm screen. The fraction $< 63\,\mu m$ was disposed, while the fraction $> 63\,\mu m$ was dried and later dry-sieved into the fractions 63–125 µm, 125–150 µm, 150–250 µm, 250–400 µm, and $> 400\,\mu m$. As with the sediment core samples, only the fraction $> 150\,\mu m$ was used for the present work.

## 2.3 Choice of species

A total of eight morphospecies of planktonic Foraminifera were used as model species for the studies presented here. Exemplary images of those species are shown in Fig. 2.2, and further information are summarized in Table 2.1. From the spinose planktonic Foraminifera the species *Globigerina bulloides* (d'Orbigny, 1826), both *Globigerinoides ruber* (d'Orbigny, 1839) morphospecies (i.e. *G. ruber* (white) and *G. ruber* (pink)), *Globigerinoides elongatus* (d'Orbigny, 1826), *Globigerinoides sacculifer* (Brady, 1877), and *Orbulina universa* (d'Orbigny, 1839) were used. From the non-spinose morphogroup the two species *Globorotalia inflata* (d'Orbigny, 1839) and *Globorotalia scitula* (Brady, 1882) were used.

The species were chosen to fulfil a variety of requirements in the respective studies. First, it was generally tried to limit the influence of cryptic speciation on the analyses (compare Sec. 1.2). Only two of the chosen morphospecies (*G. ruber* (pink) and *G. sacculifer*) are supposed to represent only one biospecies, whereas the other six either comprise several biospecies or have not been investigated concerning their genetic diversity, yet. The studies have therefore been designed in a way that limits the potential influence of cryptic speciation in such cases, by choosing sampling localities such that the community should have been dominated by (if not been composed exclusively of) a single genotype (Darling and Wade 2008, Morard et al.

2011). It must be kept in mind, however, that the current knowledge about genetic diversity in planktonic Foraminifera is far from complete, so that additional yet undiscovered genetic types could potentially exist, or already known genotypes could occur in regions where they have not been found yet. In combination with the potential of migration of genotypes over long time spans, such as thousands of years, it was not always possible to fully exclude the presence of an unresolved cryptic diversity in the samples. Those uncertainties have in each case been dealt with as good as possible, however, and the reader is referred to the individual manuscripts in Part II of this work for a case-specific discussion of this matter.

For the studies presented in Manuscripts 1–3 it was further important to cover a wide range of ecologies of the studied species, in order to test whether the same patterns occur in symbiont-bearing and symbiont-barren species as well as species inhabiting different depth habitats. Especially in the study for Manuscript 2, *G. bulloides* (symbiont-barren, cold-adapted) was chosen as a contrasting species to the warm-adapted, symbiont-bearing *G. ruber* (white) which nevertheless inhabits a comparable depth habitat and was thus subjected to comparable environmental change. In the same study, *G. elongatus* was chosen because it was traditionally considered to be a phenotype of *G. ruber* and therefore both species were often pooled for analyses in the past (Wang 2000). Here, it should be explicitly tested what effect that artificial pooling could have on calcification studies. To achieve those goals, in the species chosen for Manuscript 2 the requirement for a well-constrained hidden diversity in the populations studied had to be relaxed.

For the study presented in Manuscript 4, species with comparable environmental requirements and habitats were explicitly chosen to investigate whether the same environmental change triggers different reactions under such conditions. This should help to further unravel to what extend observed morphological changes are comparable when the inducing factors are as similar as possible.

## 2.4 Methods for biometric analyses

The biometric analyses conducted for this thesis include measurements of shell calcification and shell growth patterns. For shell calcification analyses (Manuscripts 1 and 2) foraminiferal shells were cleaned by sonication and weighed either in small groups (up to *c.*50 specimens) or as single shells in tin weighing boats using a microbalance with a high precision ($d = 0.1\,\mu g$). Afterwards, the shells were mounted on glass-slides using double-sided adhesive tape or permanent glue,

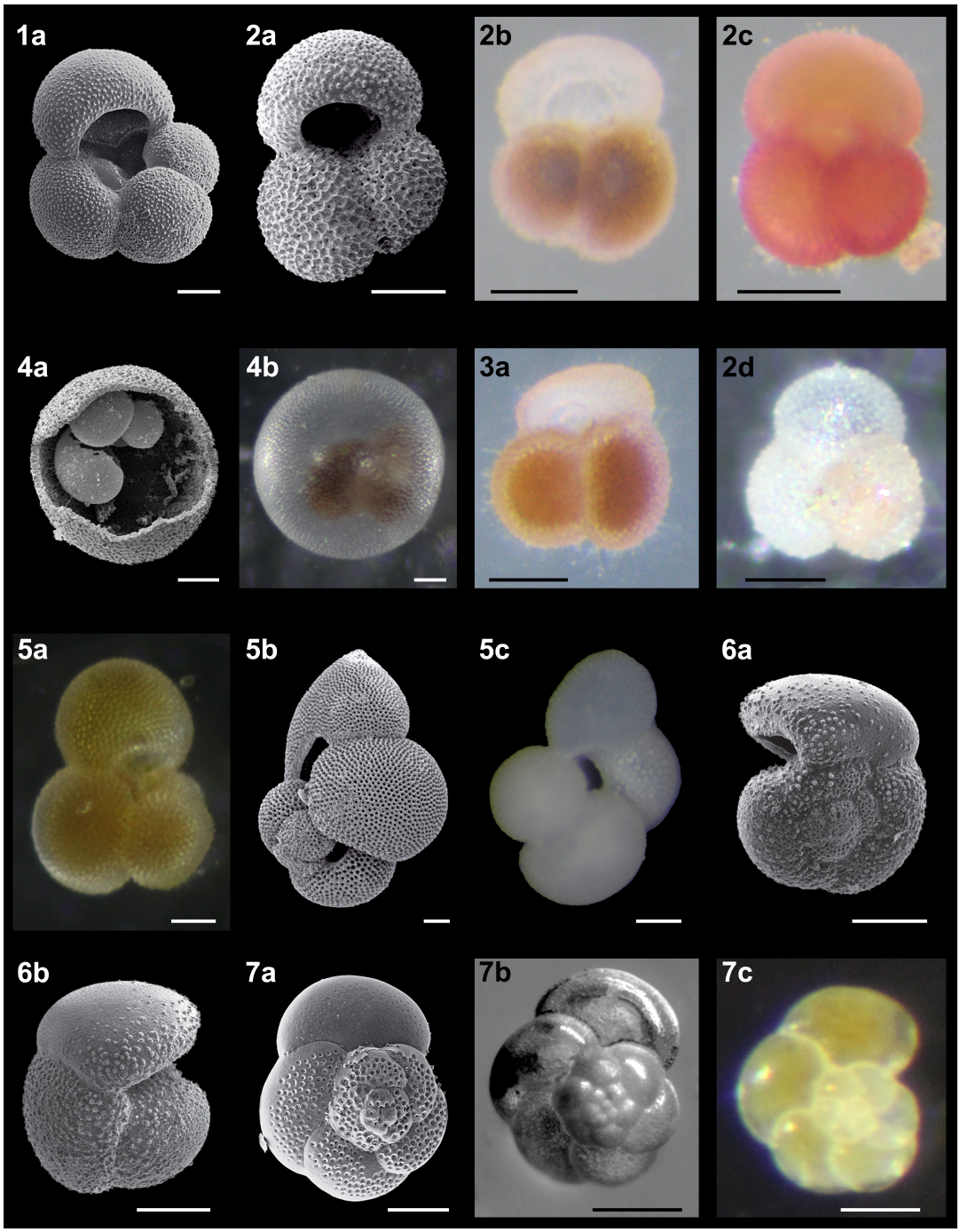
**Table 2.1** List of the morphospecies of planktonic Foraminifera used in this thesis, providing information about their morphogroup, possession of symbionts, approximated depth habitat, currently known genetic diversity, and manuscripts of the present thesis in which the species was used. Note that for *G. elongatus* no studies specifically investigating their depth habitat exist, but there is good reason to assume that this species prefers slightly deeper and cooler water masses than *G. ruber* (e.g. Steinke et al. 2005).

| Group | Species | Symbionts | Depth | Gen. div. | Man. |
|---|---|---|---|---|---|
| Spinose | *Globigerina bulloides* | − | 20–300 m[1] | 7[3] | 2 |
| | *Globigerinoides ruber* (white) | + | 20–100 m[1] | 4[4] | 2 |
| | *Globigerinoides ruber* (pink) | + | 20–100 m[1] | 1[4] | 1 |
| | *Globigerinoides elongatus* | + | 20–100 m(?) | 4[4] | 2 |
| | *Globigerinoides sacculifer* | + | 20–50 m[1] | 1[5] | 4 |
| | *Orbulina universa* | + | 20–100 m[1] | 3[6] | 1, 3, 4 |
| Non-spinose | *Globorotalia inflata* | − | 20–300 m[1] | 2[7] | 1 |
| | *Globorotalia scitula* | − | 200–500 m[2] | ? | 1, 3 |

[1]Pujol and Vergnaud Grazzini (1995), [2]Schiebel et al. (2002), [3]Darling and Wade (2008), [4]Aurahs et al. (2011), [5]André et al. (2013), [6]de Vargas et al. (1999), [7]Morard et al. (2011)

oriented in a standardized position, and photographed under a binocular microscope in transmitted light with constant magnification per species. From the photographs, shell size could be approximated by measuring the cross-sectional area of the shell.

**Figure 2.2** ***(on the next page)*** Scanning electron microscope (SEM) and light microscope (LM) images of species used in this thesis. **1:** *Globigerina bulloides* as SEM image. **2:** *Globigerinoides ruber* contains two morphospecies. *Globigerinoides ruber* (white) is characterized by a white shell (2a (SEM image), 2b (LM image, live specimen)), whereas *Globigerinoides ruber* (pink) shows an orange to red colour of the shell (2c, LM image, live specimen), although this colouring is often less prominent and restricted to ontogenetically early chambers in fossil material (2d, LM image). **3:** *Globigerinoides elongatus* with its typically flattened chambers, here shown as an LM image of a live specimen, was first recognized as a separate phenotype within the *Globigerinoides ruber* plexus (Wang 2000), but molecular analyses have shown that it is a separate biospecies (Aurahs et al. 2011). **4:** *Orbulina universa* shows a very derived morphology, where an initial trochospiral juvenile shell—which is often visible in life specimens (4b, LM image) due to the deeply coloured cytoplasm—is overgrown by a spherical terminal chamber. In the SEM the juvenile shell is only visible when the terminal chamber is cracked open (4a). **5:** *Globigerinoides sacculifer* (5a, LM image, live specimen) is known for its high phenotypic plasticity, including forms which built a sac-like terminal chamber (5b (SEM image), 5c (LM image, sedimentary specimen)). **6:** *Globorotalia inflata* as SEM images from spiral (6a) and umbilical (6b) side. **7:** *Globorotalia scitula* from spiral side as SEM image (7a), and LM images from sedimentary (7b) and live specimens (7c). Scale bars equal 100 µm. Image sources: 1a, 2a, 5b, 6a, 6b, 7a – Hesemann (2009); 2b, 2c, 3a – WG Micropalaeontology–Palaeoceanography, MARUM, Bremen; 2d, 4a, 5c, 7b – M. F. G. Weinkauf; 4b, 5a, 7c – Workgroup Mikropalaeontology–Palaeoceanography (2013)


1a
2a
2b
2c
4a
4b
3a
2d
5a
5b
5c
6a
6b
7a
7b
7c

The calcification intensity of individual shells was then calculated as area density in $\mu g\,\mu m^{-2}$ (Marshall et al. 2013), by dividing the weight by the cross-sectional area.

For shell morphological analyses (Manuscripts 3 and 4), foraminiferal shells were mounted on glass slides and photographed as described above. In dependence of the planned type of analysis either transmitted or reflected light images have been taken. Morphological parameters have been extracted directly from the images using image-analysis software. Data extraction was performed semi-automated or manually depending on the anticipated data and the software capabilities. Especially in case of manual data gathering, the resulting datasets have been analysed for the contained error using standard procedures (Yezerinac et al. 1992). Ensuing analyses with the thus derived data covered the spectrum of traditional morphometrics and geometric morphometrics. Those included quantification of shell size, shell roundness, spiral growth pattern, and shell shape descriptors. A detailed explanation of those methods is beyond the scope of this section. The reader is referred to the individual manuscripts in Part II of this thesis and the introductory standard literature (e.g. Bookstein 1991, Hammer and Harper 2006, Claude 2008, Zelditch et al. 2012).

# 3 Results and discussion

## 3.1 Summary of main results

The main objective of this thesis was a broad assessment of the influence of environmental factors on shell features of planktonic Foraminifera. It was aimed at understanding in which way shell biometry in this taxon is influenced by the environment and potentially induced by stress reactions, so that it would be useful as an environmental proxy. The analyses conducted can be roughly divided into two categories, viz. shell calcification and shell morphology. For both topics, the purely abiotic influence of sea water parameters on the measured phenotypic traits as well as the biotic component that reflects a reaction of the organism to environmental stress was investigated.

The first two manuscripts mainly deal with shell calcification, with Manuscript 2 also investigating shell size. In Manuscript 1, four species of planktonic Foraminifera, two symbiont-bearing and two symbiont-barren, from a Mediterranean sapropel were analysed concerning their shell calcification intensity. A distinct positive relationship between shell calcification intensity and stable oxygen isotope values from shells of *G. ruber* (white) was discovered amongst all four species. In the unique setting of the onset of Sapropel S5, the $\delta^{18}$O values of *G. ruber* (white) shells represent the amount of freshwater influx into the Eastern Mediterranean Sea. Therefore, the observed pattern is mainly driven by salinity, which itself is strongly correlated with carbonate saturation of the sea water. Results of this study thus imply that shell calcification in planktonic Foraminifera is indeed tightly linked to the $\Omega$ value (saturation state with respect to the solids) of the sea water across species of different habitats and trophic modes. Conversely, no impact of optimum growth conditions on shell calcification could be observed, under the assumption that optimal growth conditions are positively correlated to the abundance of the species. Neither a long-term correlation between species abundance and calcification intensity, nor short-term excursions of shell calcification in response to terminal environmental stress levels leading to local extinction could be detected.

Manuscript 2 presents results of a one-year-long sediment trap series from the Northeast Atlantic Ocean. This region is marked by a stable carbonate equilibrium system, so that it allowed to study the influence of parameters other than $\Omega$ on the shell calcification in planktonic Foraminifera. Here, three species at the edge of their respective regional distribution were investigated concerning the size–weight scaling and calcification intensity of their shells. In addition to the symbiont-barren species *G. bulloides*, this study included the two symbiont-bearing species *G. ruber* (white) and *G. elongatus*. The latter two species were traditionally considered ecophenotypes of the same species, and thus their analysis in separation as conducted in this study allowed an assessment of the potential influence of cryptic speciation on shell calcification studies. The results of the study implied that the size–weight scaling is fairly constant per species and widely unrelated to environmental factors, so that shell calcification studies can provide a reliable environmental proxy that is not subject to any second-level variation. However, the size–weight scaling is significantly different between species, so that absolute values of calcification intensity (although normalized for shell size) are not comparable between species. Both *Globigerinoides* species show a correlation of shell calcification intensity with temperature (positive) and productivity (negative). However, it could be shown that *G. ruber* (white) and *G. elongatus* exhibit different base calcification intensities and show non-parallel reaction terms in regard to environmental parameters. Furthermore, mixing both species introduces spurious deviations of their combined size–weight scaling slope. This implies that non-selective mixtures of cryptic species lead to systematic errors in shell calcification studies, that more reflect the relative abundances of the species rather than actual changes in shell calcification. Additionally, it was found that shell calcification is not correlated with species-abundance in *G. ruber* (white), that it is positively correlated with abundances in *G. elongatus*, and that it shows a negative correlation in *G. bulloides*. It must therefore be concluded, that the correlation between shell calcification intensity and optimum growth conditions in planktonic Foraminifera is highly species-specific. Furthermore it was found, that shell size is correlated with temperature and productivity in *G. bulloides*, but that no species exhibited a relationship between shell size and optimum growth conditions as inferred by species abundance.

Manuscripts 3 and 4 present results from morphometric studies. In Manuscript 3 the morphology of two species, the symbiont-bearing *O. universa* and the symbiont-barren *G. scitula*, from samples in the vicinity of Sapropel S5 in the Eastern Mediterranean Sea was studied. This period was marked by a variety of environmental

changes that led to a relatively fast and pronounced change in the habitats of the foraminifer community. The existence of long-term changes in morphology as seemingly being the result of either directional or stabilizing selection was observed. These changes were likely a response to environmental changes in relationship to the salinity and stratification overturn related to the onset of Sapropel S5. Furthermore, it was shown that unfavourable environmental conditions lead to disruptive selection, bet-hedging, and increased levels of developmental instability, all of which are processes that leave a discernible imprint in the morphology of foraminiferal communities. In both species, the community reached a unique morphological state in relation to terminal stress levels immediately before the occurrence of local extinctions.

Manuscript 4 analyses the morphological development of the two symbiont-bearing species *O. universa* and *G. sacculifer* during MIS 12 in the Red Sea, as response to drastic changes in sea water salinity. The study could show that reaction norms of morphological parameters are highly species-specific. While disruptive selection prevailed in *O. universa* during phases of increasing environmental stress, *G. sacculifer* showed trends for canalization under the same framework parameters. Moreover, only a limited link between shell morphology and environmental parameters as well as species abundance could be observed. Especially, while the abundance patterns differ dramatically, the timing of morphological changes in both investigated species is nearly synchronous. In this regard, it is remarkable that *O. universa* shows a first severe drop in abundance to nigh-extinction approximately 6000 yrs before the onset of the aplanktonic zone, without any evidence for a contemporaneous morphological deviation of the population. Both observations indicate a strong relationship between morphology and the environment in those species, that is not related to optimum growth conditions as they could be approximated by species abundances.

## 3.2 Discussion

The studies summarized in this thesis contributed significantly to answering many open questions in biometric studies of planktonic Foraminifera. As will be detailed hereafter, it was possible to enhance understanding of yet not fully understood phenomena, to identify further problems in foraminiferal biometrics, and to unravel so far unknown processes concerning foraminiferal ecology.

In agreement with several earlier studies (e.g. Lombard et al. 2011, Manno et al.

2012, Marshall et al. 2013) it could be shown that shell calcification in planktonic Foraminifera seems to be driven by the carbonate saturation state of the sea water. This relationship appears to apply uniformly throughout species of all morphogroups and trophic modes (compare Manuscript 1). However, when eliminating the influence of carbonate saturation, still a large environmental influence on calcification intensity of foraminiferal shells could be observed (Manuscript 2). It can be assumed that this influence is always present, and modifies shell calcification reactions even if carbonate saturation is the main driving factor (Manno et al. 2012).

While the influence of carbonate saturation seems to have the same effect on all species, other environmental parameters differ species-specific in the direction of their impact on shell calcification intensity. More troublesome, the effects exercised by some parameters are not even intuitive. While de Villiers (2004) proposed a universal positive correlation between optimum growth conditions and shell calcification intensity, the works included in this thesis do not support this idea (Manuscripts 1 and 2). Theoretically higher calcification intensities would be expected under more favourable environmental conditions. Since shell calcification is an energy-consuming process (Spero 1988) it likely participates in a trade-off of energy allocation within the cell. More suitable environmental conditions should provide more energy for the cell and consequently also more energy that can be channelled into calcification, since more energy should be available for the cell under such conditions. Nevertheless, during the deposition of Sapropel S5 (Manuscript 1) neither a general correlation between shell calcification intensity and species abundance nor a calcification reaction to terminal stress levels could be observed. Sediment trap samples from the northeast Atlantic Ocean (Manuscript 2) draw a similar picture, with no correlation between shell calcification intensity and abundance of *G. ruber* (white). While shell calcification and abundance are indeed positively correlated in *G. elongatus* in the same samples, a negative correlation between shell calcification and abundance in *G. bulloides* could be observed. This replicates results by Aldridge et al. (2012), making it unlikely to be an exceptional case for that species.

Taking into account that shell sizes in the samples from sediment trap L1/K276 also did not show any correlation with either absolute or relative abundances, it is questionable whether or not species abundance is a reasonable proxy for optimal growth conditions at all. This idea is further perpetuated by the complete lack of morphological changes in *O. universa* during a first significant drop in abundance before the onset of the aplanktonic zone in the Red Sea (Manuscript 4). Under the assumption that population sizes are highly variable at all times (Ludwig 1999),

it might therefore be complicated to assess environmental suitability by species abundance alone.

On the other hand it could be shown that cryptic speciation and non-constant mixing of different biospecies introduces large systematic errors into shell calcification studies, and probably morphological studies as well (compare Morard et al. 2009, Morard et al. 2011, Weiner et al. 2015). While this influence could be shown in the case of *G. ruber* (white)/*G. elongatus* in the northeast Atlantic, it could not be controlled for in *G. bulloides* from the same samples. Therefore, it is possible that the patterns observed by Aldridge et al. (2012) and in Manuscript 2 are both the result of such mixing of cryptic species. Nevertheless, even with this possibility in mind, the problem of cryptic speciation was shown to be severe in biometric studies on planktonic Foraminifera. Studies that use non-selective mixtures of different species (e.g. de Moel et al. 2009, Naik et al. 2013) might therefore be highly susceptible for spurious patterns in their data, leading to misconceptions and complicating the interpretation of biometric markers. A prominent example for this is the species *Globorotalia truncatulinoides*, where the height–width ratio of the shell had been suggested as palaeoceanographic proxy for temperature (Healy-Williams and Williams 1981, Lohmann and Malmgren 1983, Healy-Williams 1983/84, Healy-Williams et al. 1985) until it was shown that this morphological difference corresponds to pseudo-cryptic speciation with distinct distribution patterns of the different biospecies (de Vargas et al. 2001, Renaud and Schmidt 2003). Another example is the coiling-direction in *Neogloboquadrina pachyderma*, which was supposed to represent a valuable morphometric proxy (e.g. Ericson 1959, Kennett 1968b). Later, molecular analyses revealed that the old concept of *N. pachyderma*, on which this proxy was based, combines two different biospecies (*N. pachyderma* and *N. incompta*) with inherently different coiling-directions (Darling et al. 2006).

The selective patterns in shell morphology documented in this thesis are to a large degree exceptional considering that few studies have so far assessed morphological parameters other than shell size and coiling direction in planktonic Foraminifera. And even for those parameters results obtained by earlier studies are controversial (compare Table 3.1). On the one hand, morphological reactions seem to vary species-specifically, and different species can exhibit completely opposite patterns when exposed to virtually identical environmental conditions (Manuscript 4). Given that different species occupy different ecological niches that is what one would expect, especially when reactions are driven by the environmental factors instead of the stress reaction itself. On the other hand, reactions within the same species seem to be

rather universal. For example, the negative correlation of shell size with temperature in *G. bulloides* has already been observed before (Malmgren and Kennett 1976, Manuscript 2). Such a reaction would be expected from a species like *G. bulloides* that favours lower temperatures, if shell size is correlated with environmental suitability. Remarkably, all studies inferring optimal growth conditions directly seem to confirm this view, while only studies where optimal growth conditions were approximated by abundance data disagree (compare Table 3.1). This is yet another hint, supported by Manuscripts 2–4, that species abundance is not a reliable indicator for optimal environmental conditions and should be abandoned for that purpose in the future.

A comparison of the other morphometric trends reported in this thesis is not as straightforward. Many of these analyses have rarely or not at all been applied to planktonic Foraminifera before. The abundance of abnormal morphotypes in *O. universa* has been reported to be correlated with environmental stress before (Caron et al. 1987), but also to be positively correlated with productivity (Robbins 1988). One could argue that increased productivity leads to lower penetration depths of the light into the water column due to the higher algae density, thus inducing environmental stress in a symbiont-bearing species like *O. universa*. But this does not explain the lack of abnormal morphotypes in the Red Sea samples in Manuscript 4. It is, however, consistent with the assumption that increased productivity leads to increases of the abundance of abnormal morphotypes (Robbins 1988), while stress itself if induced by other environmental parameters does not. This would, however, contradict results by Caron et al. (1987), where abnormal morphotypes were induced by salinity changes. It might be that the generally very low abundances of abnormal morphotypes in the Red Sea dispersed an existing pattern, that therefore could not be discovered.

Shell roundness in *O. universa* shows a fairly consistent pattern, indicating unfavourable environmental conditions irrespective of the particular parameter responsible. It normally decreases when stress levels arguably increase (higher productivity in Manuscript 3, higher salinity in Manuscript 4). In this regard it is interesting, that at the same time the variation of shell roundness increased in the Red Sea samples (Manuscript 4) but not the Mediterranean samples (Manuscript 3).

**Table 3.1** Representative summary of results from earlier works investigating the impact of environmental factors on planktonic foraminifer morphology. The majority of the studies deals with shell size, with only a minority studying other morphological parameters. For shell porosity the review is limited to such studies which at least partly tried to understand the environmental influence on porosity, disregarding those which solely aim at using porosity differences for species differentiation. For coiling direction only a few selected examples were cited because too many studies exist on that topic to fit within the framework of this thesis (compare Hemleben et al. 1989) and their results have always been rather comparable.

| Symbionts | Species | Publication | Material type | Phenotypic trait | Temp. | Sal. | Prod. | Optimum growth cond. | Other |
|---|---|---|---|---|---|---|---|---|---|
| NA | Assemblage | Bé (1968) | Life Foraminifera | Porosity | + | | | | |
| NA | | Hecht (1976) | Life Foraminifera | Shell size | | | | + | |
| NA | | Kennett (1976) | NA | Shell size | | | | + | |
| NA | | Schmidt et al. (2003) | Fossil sediment | Shell size | | | − | + | |
| NA | | Schmidt et al. (2004) | Fossil sediment | Shell size | | | + | + | + with stratification |
| NA | *S. linaperta* | MacLeod (1990) | Fossil sediment | Shape | | | | | Progenesis with abundance increase and habitat change |
| NA | | MacLeod et al. (1990) | Fossil sediment | Shape | | | | | Progenesis with abundance increase and habitat change |
| NA | | MacLeod et al. (2000) | Fossil sediment | Shape | | | | | Progenesis with abundance increase and habitat change |
| NA | | MacLeod (1990) | Fossil sediment | Shell size | | | | $-^{b}$ | |
| NA | | MacLeod et al. (1990) | Fossil sediment | Shell size | | | | $-^{b}$ | |
| NA | | MacLeod et al. (2000) | Fossil sediment | Shell size | | | | $-^{b}$ | |
| + | *G. glutinata* | Naidu and Malmgren (1995) | Fossil sediment | Shell size | | | | | + with upwelling |
| + | | Ortiz et al. (1995) | Plankton net samples | Shell size | | | − | | |
| + | *G. ruber** | Hecht (1974) | Surface sediment | Chamber expansion | 0 | − | | | + with latitude |
| + | | Naidu and Malmgren (1995) | Fossil sediment | Shell size | | | | | + with upwelling |
| + | | Ortiz et al. (1995) | Plankton net samples | Shell size | + | | − | | |
| + | | Hecht (1974) | Surface sediment | Shell size variation | + | − | | | |
| + | *G. ruber* (white) | **This work, Manuscript 2** | Sediment trap samples | Shell size | 0 | | 0 | $0^{b}$ | |
| + | *G. elongatus* | **This work, Manuscript 2** | Sediment trap samples | Shell size | 0 | | 0 | $0^{b}$ | |
| + | *N. dutertrei* | Naidu and Malmgren (1995) | Fossil sediment | Shell size | | | | | + with upwelling |
| + | | Ortiz et al. (1995) | Plankton net samples | Shell size | | | − | | |
| + | *G. sacculifer* | Hecht (1974) | Surface sediment | Chamber expansion | 0 | 0 | | | 0 with latitude |
| + | | **This work, Manuscript 4** | Fossil sediment | Antisymmetry of coiling | | − | | $-^{b}$ | |
| + | | **This work, Manuscript 4** | Fossil sediment | Disparity | | − | | $+^{b}$ | |
| + | | Hemleben et al. (1987) | Laboratory experiments | Shell size | + | + | | | |
| + | | **This work, Manuscript 4** | Fossil sediment | Shell size | | − | | $+^{b}$ | |
| + | | Hecht (1974) | Surface sediment | Shell size variation | 0 | 0 | | | 0 with latitude |
| + | | **This work, Manuscript 4** | Fossil sediment | Shell size variation | | + | | $+^{b}$ | disruption with stress |
| + | *O. universa* | Caron et al. (1987) | Laboratory experiments | Abnormal morphotypes abundance | | | | − | + with stress |
| + | | Robbins (1988) | Surface sediment | Abnormal morphotypes abundance | | | + | | |
| + | | **This work, Manuscript 3** | Fossil sediment | Abnormal morphotypes abundance | | | | $-^{b}$ | + with stress, + with sapropel onset |
| + | | **This work, Manuscript 4** | Fossil sediment | Abnormal morphotypes abundance | | | | $0^{b}$ | |
| + | | Colombo and Cita (1980) | Fossil sediment | Porosity | | | | | questionable with climate |
| + | | **This work, Manuscript 3** | Fossil sediment | Shell roundness | | | | | 0 with stress, − with sapropel onset |
| + | | **This work, Manuscript 4** | Fossil sediment | Shell roundness | | 0 | | $+^{b}$ | |
| + | | **This work, Manuscript 3** | Fossil sediment | Shell roundness variation | | | | | 0 with stress, 0 with sapropel onset |
| + | | **This work, Manuscript 4** | Fossil sediment | Shell roundness variation | | + | | $-^{b}$ | |
| + | | Bé and Duplessy (1976) | Fossil sediment | Shell size | + | | | | |
| + | | Bé et al. (1973) | Plankton net samples and surface sediment | Shell size | + | | | | |
| + | | Caron et al. (1987) | Laboratory experiments | Shell size | + | | | | |
| + | | Colombo and Cita (1980) | Fossil sediment | Shell size | | | | | + with climate (lagged) |
| + | | Haenel (1987) | Fossil sediment | Shell size | + | − | | | |
| + | | Malmgren and Healy-Williams (1978) | Fossil sediment | Shell size | | | | | No clear correlation with palaeoclimate |
| + | | Ortiz et al. (1995) | Plankton net samples | Shell size | | | − | | |
| + | | **This work, Manuscript 3** | Fossil sediment | Shell size | | | | | 0 with stress, 0 with sapropel onset |
| + | | **This work, Manuscript 4** | Fossil sediment | Shell size | | 0 | | $+^{b}$ | |
| + | | **This work, Manuscript 3** | Fossil sediment | Shell size variation | | | | | 0 with stress, − with sapropel onset |
| + | | **This work, Manuscript 4** | Fossil sediment | Shell size variation | | | | | bimodal with stress |
| − | *G. bulloides* | Malmgren and Kennett (1976) | Surface sediment | Kummerforms | | | | − | |
| − | | Malmgren and Kennett (1976) | Surface sediment | Shell size | − | | | $+^{b}$ | |
| − | | Malmgren and Kennett (1978b) | Fossil sediment | Shell size | − | | | | |
| − | | Malmgren and Kennett (1978a) | Fossil sediment | Shell size | − | | | | |
| − | | Naidu and Malmgren (1995) | Fossil sediment | Shell size | | | | | + with upwelling |
| − | | Boltovskoy (1973) | Life Foraminifera(?) | Sinistral coiling | − | | | | |
| − | | Malmgren and Kennett (1976) | Surface sediment | Sinistral coiling | − | | | | |
| − | | Naidu and Malmgren (1996) | Fossil sediment | Sinistral coiling | | | + | | |
| − | | **This work, Manuscript 2** | Sediment trap samples | Shell size | − | | + | $0^{b}$ | |
| − | *G. siphonifera/G. calida/G. radians* | Weiner et al. (2015) | Life Foraminifera | Porosity | | | | | Provincialism |
| − | *G. scitula* | Baumfalk et al. (1987) | Fossil sediment | Chamber lobateness | + | | | | |
| − | | Baumfalk et al. (1987) | Fossil sediment | Chamber number | − | | | | |
| − | | Vasicek (1953) | Fossil sediment | Coiling direction | | | | | selection by environmental factors |
| − | | **This work, Manuscript 3** | Fossil sediment | Sinistral coiling | | | | $0^{b}$ | 0 with stress |
| − | | **This work, Manuscript 3** | Fossil sediment | Growth regularity | | | | $-^{b}$ | − with stress |
| − | | Baumfalk et al. (1987) | Fossil sediment | Kummerforms | | | | | + with reproductive stress |
| − | | **This work, Manuscript 3** | Fossil sediment | Kummerforms | | | | $0^{b}$ | 0 with stress |
| − | | Baumfalk et al. (1987) | Fossil sediment | Porosity | | − | | | |
| − | | Itou et al. (2001) | Life Foraminifera and sediment trap samples | Porosity | | | | | − with carbonate saturation state |
| − | | **This work, Manuscript 3** | Fossil sediment | Shell size | | | | $0^{b}$ | 0 with stress |
| − | *G. truncatulinoides* | Ericson et al. (1955) | Fossil sediment | Coiling direction | | | | | Provincialism, selection by environmental factors |
| − | | Lohmann and Malmgren (1983) | Surface sediment | Degree of conic shape and spiral convexity[a] | + | | | | |
| − | | Renaud and Schmidt (2003) | Fossil sediment | Degree of conic shape and spiral convexity[a] | + | | | | pseudo−cryptic species |
| − | | Healy-Williams (1983/84) | Surface and fossil sediment | Degree of conic shape[a] | | | | | − with latitude |
| − | | Healy-Williams and Williams (1981) | Surface sediment | Degree of conic shape[a] | | | | | − with latitude |
| − | | Healy-Williams et al. (1985) | Surface sediment | Degree of conic shape[a] | | | | | − with latitude |
| − | | Kennett (1968a) | Surface sediment | Lateral shell compression[a] | − | | | | |
| − | | Takayanagi et al. (1968) | NA | Lateral shell compression[a] | − | | | | |
| − | | Kennett (1969) | Surface sediment | Sinistral coiling | − | | | | |
| − | *N. incompta* | Moller et al. (2013) | Surface sediment | Shell size | + | | | | |
| − | | Ortiz et al. (1995) | Plankton net samples | Shell size | | | 0 | | |
| − | *N. pachyderma* | Moller et al. (2013) | Surface sediment | Chamber lobateness | 0 | | | | |
| − | | Moller et al. (2013) | Surface sediment | Shell roundness | 0 | | | | |
| − | | Moller et al. (2013) | Surface sediment | Shell size | − | | | | |
| − | *N. pachyderma*** | Kennett (1968b) | Surface sediment | Chamber number | | | | | highest in intermediate latitutudes |
| − | | Dow (1977) | Fossil sediment | Porosity | + | | | | |
| − | | Kennett (1968b) | Surface sediment | Shell thickness | | | | | −towards north |

While this could be the result of different forcing of the stress reaction, another explanation is also perceivable. The shell size and shell roundness could be shown to correlate significantly in the Red Sea *O. universa* specimens, mirroring the bimodality observed in the shell size data alone. This bimodality could also be responsible for the observed increase in roundness variance, and could be explained in two ways. Either, there is a threshold size below which the terminal chamber in *O. universa* cannot be built nearly spherical due to geometrical limitations enforced by the juvenile shell which has to fit within the sphere. This explanation is supported by the fact, that the smallest specimens from the Red Sea (Fig. 7.4) are much smaller than those from the Mediterranean (Fig. 6.5), and that the subgroup with lower variation in the Red Sea corresponds well to the specimens observed in the Mediterranean (Fig. 7.10). Alternatively, the two sub-populations encountered in the Red Sea could represent two different biospecies. While at present in both the Mediterranean Sea and the Red Sea only one biospecies of *O. universa* is known to occur (Darling and Wade 2008) this does not exclude the possibility that other species were present in the past. If this hypothesis should be true, it would be the first instance that *O. universa* pseudo-cryptic species can be differentiated by other means than porosity and shell thickness (Morard et al. 2009). However, the increasing abundance over time of the morphotype which would then have to be the invader species makes this unlikely. If another species from the open-marine Indian Ocean would have invaded the Red Sea at that time it is imperceivable that it would have been better adapted to the persistently very high salinity levels during this phase. A purely morphological reaction within a homogeneous community is thus the much more likely explanation for observed roundness changes in the *O. universa* community.

Of special interest are the developmental patterns that can be observed across species, and that are seemingly worthwhile candidates for further research. A variety of selective patterns could be observed in different species when exposed to environmental stress, which most interestingly tend to vary between species under the same conditions although their environmental requirements are comparable. While canalization seems to dominate the *G. sacculifer* community from MIS 12 in the Red Sea when salinity increases, at the same time *O. universa* exhibits decanalization (Manuscript 4). Comparably, the same environmental change that leads to bet-hedging (and thus increase in variance) in *G. scitula* also yields canalization of shell size in *O. universa* (Manuscript 3). It is reasonable to assume different modes of selection in species with varying environmental preferences occupying different niches, when exposed to the same kind of environmental change. Nevertheless, at

**Table 3.2** Summary of apparent relationships between biometric and environmental parameters, as inferred from studies included in this thesis.

| Species | Biometric parameter | Indication |
|---|---|---|
| *G. ruber* (white) | Calcification intensity | Temperature |
| *G. ruber* (pink) | Calcification intensity | Carbonate saturation |
| *G. elongatus* | Calcification intensity | Temperature, optimal environment |
| *G. sacculifer* | Sacculifer-morphotype | Favourable environment |
| | Antisymmetry | Environmental stress/salinity |
| | Canalization | Environmental stress/salinity |
| *O. universa* | Calcification intensity | Carbonate saturation |
| | Incidence of abnormal morphotypes | Productivity/terminal stress(?) |
| | Shell size variation | Vertical circulation(?) |
| | Shell roundness | Environmental stress |
| *G. bulloides* | Calcification intensity | Optimal environment (neg.) |
| | Shell size | Temperature, productivity |
| *G. scitula* | Calcification intensity | Carbonate saturation |
| | Growth irregularity | Terminal Stress |
| *G. inflata* | Calcification intensity | Carbonate saturation |

least part of the observed trends, e.g. disruptive selection in shell size in *O. universa*, seem to be linked to particular environmental changes, for instance salinity changes. A summary of all biometric parameters inferred from the studies included in this thesis is shown in Table 3.2.

Most remarkably, regardless of the exact kind of reaction actually shown, several communities exposed to terminal stress levels leading to extinction showed prominent morphological changes in association with raising levels of environmental stress. Such reactions, ranging from disruptive selection and bet-hedging to stabilizing selection and canalization occurred in some way in all species investigated in the manuscripts comprising this thesis. We can therefore assume that such patterns, leading to a unique state of the population morphology, can be used to deduce stress levels and to predict future extinctions. Admittedly, the type of reaction differs not only between species, but also between different populations of the same morphospecies, as evidenced by *O. universa* from the Mediterranean Sea and Red Sea (Manuscripts 3 and 4). Further elaboration is needed to fully understand those selective patterns in planktonic Foraminifera and to make them broadly applicable as proxy for environmental reconstructions and monitoring in recent environments.

Once those patterns are better understood, we can use them as an alternative to population dynamics (which suffer from the naturally large variability of population sizes, compare discussion above) to predict extinctions and to better understand evolutionary and selective patterns that lead to (regional) extinctions of planktonic Foraminifera.

In conclusion this work could show that many biometric patterns in planktonic Foraminifera are mainly influenced by environmental parameters. Some of those relationships have already been described before and could be confirmed here, while other previously postulated relationships cannot be supported by the present work.

The effect of cryptic speciation and a hidden diversity in planktonic Foraminifera must be considered with great caution in biometric studies. There have been examples in the past were promising environmental proxies turned out to be the result of misconceptions concerning the hidden diversity within a morphospecies. Within this thesis, some more examples of such pitfalls could be discovered, so that they can be hopefully avoided in the future. Nevertheless, there are more potential candidates for such problems, and some uncertainties concerning the interpretation of observed patterns can also be found within the present work.

The work could show that a variety of biometric markers exist that can be used to reconstruct past environmental conditions, assess levels of environmental stress a community was exposed to, and even predict impending extinctions. It could be shown that many of the selective patterns that govern evolution in multicellular animals can already be observed in planktonic Foraminifera, and that they likely also had an influence on the evolvability of that taxon. This thesis, however, also serves as an example that the situation in planktonic Foraminifera is equally difficult to that in many other taxa that have yet been extensively studied biometrically, and that much more work is needed to unravel the mechanisms behind those patterns.

### 3.2.1 Future prospects

The results of several previous studies and also from this thesis indicate a large potential for biometric analyses on planktonic Foraminifera. Alas, except for studies concerning size and coiling direction not much effort was put into that field, especially during recent years. For instance, only approximately one shell calcification study per year has yet been published worldwide, although several studies have shown the value of foraminiferal shell calcification as environmental proxy. For morphometric studies this value is even lower when disregarding studies that

exclusively focus on shell size or coiling direction (Table 3.1).

Many studies have shown, that certain biometric parameters are valuable environmental proxies (e.g. Malmgren and Kennett 1976, Baumfalk et al. 1987, Moller et al. 2013, Manuscripts in this thesis), but more studies are needed to unravel their full potential. Particularly, there is need to (1) further investigate the effect of environmental factors other than $CO_3^{2-}$ in mediating shell calcification, (2) investigate further species under comparable conditions to gain more insights into species-specific biometric reactions, and (3) consolidate our knowledge about the role environmental change and stress plays in biometric reactions and how these reactions are different in dependence of the stressor. If these goals can be achieved, it is perceivable that with time and further research, biometric proxies could potentially aid in judging the applicability of other analyses (such as isotope analyses) on particular samples, or replace more traditional proxies in situations where they are not useful for the samples in question (e.g. because of contamination of isotopic signals or selective dissolution which prevents the use of transfer functions).

Furthermore, our understanding of phenotypic plasticity and factors that influence selection on phenotypic traits (e.g. Wagner 1996, Debat and David 2001, Klingenberg 2003) as well as our ability to analyse morphology (compare Adams et al. 2004) have significantly increased during the past decades, calling for a resurrection of morphometric studies on planktonic Foraminifera for phylogenetic studies. The field of molecular biology on Foraminifera has made large advances during the last decades (e.g. Pawlowski 2000), but while the taxonomy of extant species is nearly unravelled meanwhile, their phylogenetic pathways are partly still in question. Building on traditional models for speciation in the plankton (Norris 2000), biometric analyses in combination with molecular biology are potentially very useful in further elucidating how species of planktonic Foraminifera evolve.

This thesis tried to ascertain yet another part of the mechanism behind shell biometry in planktonic Foraminifera, and to further our understanding of the imprint environmental stress leaves in the growth pattern and shell symmetry of planktonic Foraminifera. It thus stands in line with many studies that have done so in the past, hopefully lending some ideas to like-minded approaches in the future.

# References


Adams, D. C., Rohlf, F. J., and Slice, D. E. (2004) Geometric morphometrics: Ten years of progress following the 'revolution', *Italian Journal of Zoology* 71: 5–16.

Aldridge, D., Beer, Ch. J., and Purdie, D. A. (2012) Calcfication in the planktonic Foraminifera *Globigerina bulloides* linked to phosphate concentrations in surface waters of the North Atlantic Ocean, *Biogeosciences* 9: 1725–39, doi:10.5194/bg-9-1725-2012.

Allen, R. D. (1964) Cytoplasmic streaming and locomotion in marine Foraminifera, In Allen, R. D. and Kamiya, N. (eds.), *Primitive Motile Systems in Cell Biology*, (London: Academic Press), pp. 407–32.

Alve, E. (1991) Benthic Foraminifera in sediment cores reflecting heavy metal pollution in Sørfjord, western Norway, *Journal of Foraminiferal Research* 21 (1): 1–19, doi:10.2113/gsjfr.21.1.1.

Alve, E. (1995) Benthic foraminiferal responses to estuarine pollution: A review, *Journal of Foraminiferal Research* 25 (3): 190–203, doi:10.2113/gsjfr.25.3.190.

Amante, C. and Eakins, B. W. (2009) ETOPO1 *1 Arc-Minute Global Relief Model: Procedures, Data Sources and Analysis*, NOAA Technical Memorandum NESDIS NGDC-24, National Geophysical Data Center, 25 pp., `http://www.ngdc.noaa.gov/mgg/global/relief/ETOPO1/docs/ETOPO1.pdf`.

Anderson, O. R. and Bé, A. W. H. (1976) A cytochemical fine structure study of phagotrophy in a planktonic foraminifer, *Hastigerina pelagica* (d'Orbigny), *Biological Bulletin* 151 (3): 437–49.

André, A., Weiner, A., Quillévéré, F., Aurahs, R., Morard, R., Douady, Ch. J., de Garidel-Thoron, Th., Escarguel, G., de Vargas, C., and Kučera, M. (2013) The cryptic and the apparent reversed: Lack of genetic differentiation within the morphologically diverse plexus of the planktonic foraminifer *Globigerinoides sacculifer*, *Paleobiology* 39 (1): 21–39, doi:10.1666/0094-8373-39.1.21.

Armstrong, H. A. and Brasier, M. D. (2005) *Microfossils*, 2nd ed., (Malden, Oxford, Carlton: Blackwell Publishing), 296 pp.

Aurahs, R., Göker, M., Grimm, G. W., Hemleben, V., Hemleben, Ch., Schiebel, R., and Kučera, M. (2009) Using the multiple analysis approach to reconstruct phylogenetic relationships among planktonic Foraminifera from highly divergent and length-polymorphic SSU rDNA sequences, *Bioinformatics and Biology Insights* 3: 155–77, `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2808177/`.

Aurahs, R., Treis, Y., Darling, K., and Kučera, M. (2011) A revised taxonomic and phylogenetic concept for the planktonic foraminifer species *Globigerinoides ruber* based on molecular and morphometric evidence, *Marine Micropaleontology* 79: 1–14, doi:10.1016/j.marmicro.2010.12.001.

Ballent, S. C. and Carignano, A. P. (2008) Morphological abnormalities in Late Cretaceous and Early Paleocene foraminifer tests (northern Patagonia, Argentina), *Marine Micropaleontology* 67: 288–96, doi:10.1016/j.marmicro.2008.02.003.

Barker, S. and Elderfield, H. (2002) Foraminiferal calcification response to glacial–interglacial changes in atmospheric $CO_2$, *Science* 297: 833–6, doi:10.1126/science.1072815.

Baumfalk, Y. A., Troelstra, S. R., Ganssen, G., and Van Zanen, M. J. L. (1987) Phenotypic variation of *Globorotalia scitula* (Foraminiferida) as a response to Pleistocene climatic fluctuations, *Marine Geology* 75: 231–40, doi:10.1016/0025-3227(87)90106-X.

Bé, A. W. H. (1968) Shell porosity of recent planktonic Foraminifera as a climatic index, *Science* 161 (3844): 881–4, doi:10.1126/science.161.3844.881.

Bé, A. W. H. (1977) An ecological, zoogeographic and taxonomic review of recent planktonic Foraminifera, In Ramsay, A. T. S. (ed.), *Oceanic Micropalaeontology*, no. i, (London, New York, San Francisco: Academic Press), chap. 1, pp. 1–100.

Bé, A. W. H. and Duplessy, J.-C. (1976) Subtropical convergence fluctuations and Quaternary climates in the middle latitudes of the Indian Ocean, *Science* 194: 419–22, doi:10.1126/science.194.4263.419.

Bé, A. W. H., Harrison, S. M., and Lott, L. (1973) *Orbulina universa* d'Orbigny in the Indian Ocean, *Micropaleontology* 19 (2): 150–92, doi:10.2307/1485162.

Bé, A. W. H., Hemleben, Ch., Anderson, O. R., and Spindler, M. (1979) Chamber formation in planktonic Foraminifera, *Micropaleontology* 25 (3): 294–307, http://www.jstor.org/stable/1485304.

Bé, A. W. H. and Hutson, W. H. (1977) Ecology of planktonic Foraminifera and biogeographic patterns of life and fossil assemblages in the Indian Ocean, *Micropaleontology* 23 (4): 369–414, doi:10.2307/1485406.

Bé, A. W. H., Spero, H. J., and Anderson, O. R. (1982) Effects of symbiont elimination and reinfection on the life processes of the planktonic foraminifer *Globigerinoides sacculifer*, *Marine Biology* 70: 73–86, doi:10.1007/BF00397298.

Beasley, D. A. E., Bonisoli-Alquati, A., and Mousseau, T. A. (2013) The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: A meta-analysis, *Ecological Indicators* 30: 218–26, doi:10.1016/j.ecolind.2013.02.024.

Bijma, J., Spero, H. J., and Lea, D. W. (1999) Reassessing foraminiferal stable isotope geochemistry: Impact of the oceanic carbonate system (experimental results), In Fischer, G. and Wefer, G. (eds.), *Use of Proxies in Paleoceanography: Examples from the South Atlantic*, (Berlin, Heidelberg: Springer-Verlag), pp. 489–512.

Boltovskoy, E. (1973) Note on the determination of absolute surface water paleotemperature by means of the foraminifer *Globigerina bulloides* d'Orbigny, *Paläontologische Zeitschrift* 47 (3–4): 152–5, doi:10.1007/BF02985703.

Boltovskoy, E., Scott, D. B., and Medioli, F. S. (1991) Morphological variations of benthic foraminiferal tests in response to changes in ecological parameters: A review, *Journal of Paleontology* 65 (2): 175–85, http://www.jstor.org/stable/1305752.

Bookstein, F. L. (1991) *Morphometric Tools for Landmark Data: Geometry and Biology*, (Cambridge, New York, Port Chester, Melbourne, Sydney: Cambridge University Press), 455 pp.

Bookstein, F. L. (2013) Random walk as a null model for high-dimensional morphometrics of fossil series: Geometrical considerations, *Paleobiology* 39 (1): 52–74, doi:10.1666/0094-8373-39.1.52.

Brady, H. B. (1877) Supplementary note on the Foraminifera of the Chalk(?) of the New Britain Group, *Geological Magazine* 4 (12): 535.

Brady, H. B. (1882) Report on the Foraminifera, In Tizard and Murray, J. (eds.), *Exploration of the Faröe Channel during the Summer of 1880, in Her Majesty's Hired Ship "Knight Errant"*, vol. 11, Proceedings of The Royal Society of Edinburgh no. cxi, (Edinburgh: The Royal Society of Edinburgh), pp. 708–17.

Breno, M., Leirs, H., and Van Dongen, S. (2011) No relationship between canalization and developmental stability of the skull in a natural population of *Mastomys natalensis* (Rodentia: Muridae), *Biological Journal of the Linnean Society* 104 (1): 207–16, doi:10.1111/j.1095-8312.2011.01702.x.

Breuker, C. J., Patterson, J. S., and Klingenberg, Ch. P. (2006) A single basis for developmental buffering of *Drosophila* wing shape, *PLOS ONE* 1 (1): e7, doi:10.1371/journal.pone.0000007.

Broecker, W. and Clark, E. (2001) An evaluation of Lohmann's Foraminifera weight dissolution index, *Paleoceanography* 16 (5): 531–4, doi:10.1029/2000PA000600.

Brummer, G. J. A. and Kroon, D. (1988) *Planktonic Foraminifers as Tracers of Ocean-Climate History: Ontogeny, Relationships and Preservation of Modern Species and Stable Isotopes, Phenotypes and Assemblage Distribution in Different Water Masses*, (Amsterdam: Free University Press), 346 pp.

Bull, J. J. (1987) Evolution of phenotypic variance, *Evolution: International Journal of Organic Evolution* 41 (2): 303–15, http://www.jstor.org/stable/2409140.

Bumpus, H. C. (1898) The elimination of the unfit as illustrated by the introduced sparrow *Passer domesticus*, *Biological Lectures Delivered at the Marine Biological Laboratory of Wood's Hole* 11: 209–26.

Burone, L., Venturini, N., Sprechmann, P., Valente, P., and Muniz, P. (2006) Foraminiferal responses to polluted sediments in the Montevideo coastal zone, Uruguay, *Marine Pollution Bulletin* 52: 61–73, doi:10.1016/j.marpolbul.2005.08.007.

Cane, T., Rohling, E. J., Kemp, A. E. S., Cooke, S., and Pearce, R. B. (2002) High-resolution stratigraphic framework for Mediterranean Sapropel S5: Defining temporal relationships

between records of Eemian climate variability, *Palaeogeography, Palaeoclimatology, Palaeoecology* 183: 87–101, doi:10.1016/S0031-0182(01)00461-8.

Caron, D. A., Bé, A. W. H., and Anderson, O. R. (1982) Effects of variations in light intensity on life processes of the planktonic foraminifer *Globigerinoides sacculifer* in laboratory culture, *Journal of the Marine Biological Association of the United Kingdom* 62 (2): 435–51, doi:10.1017/S0025315400057374.

Caron, D. A., Countway, P. D., Jones, A. C., Kim, D. Y., and Schnetzer, A. (2012) Marine protistan diversity, *Annual Review of Marine Science* 4: 467–93, doi:10.1146/annurev-marine-120709-142802.

Caron, D. A., Faber Jr., W. F., and Bé, A. W. H. (1987) Growth of the spinose planktonic foraminifer *Orbulina universa* in laboratory culture and the effect of temperature on life processes, *Journal of the Marine Biological Association of the United Kingdom* 67 (2): 343–58, doi:10.1017/S0025315400026655.

Clarke, G. M. (1993) Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality, *Environmental Pollution* 82: 207–11, doi:10.1016/0269-7491(93)90119-9.

Claude, J. (2008) *Morphometrics with R*, Use R!, (New York: Springer-Verlag), 316 pp.

Coccioni, R. (2000) Benthic Foraminifera as bioindicators of heavy metal pollution: A case study from the Goro Lagoon (Italy), In Martin, R. E. (ed.), *Environmental Micropaleontology: The Application of Microfossils to Environmental Geology*, Topics in Geobiology no. xv, (New York, Boston, Dordrecht, London, Moscow: Kluwer Academic/Plenum Publishers), chap. 3, pp. 71–103.

Colombo, M. R. and Cita, M. B. (1980) Changes in size and test porosity of *Orbulina universa* d'Orbigny in the Pleistocene record of Cape Bojador (DSDP Site 397, eastern North Atlantic), *Marine Micropaleontology* 5: 13–29, doi:10.1016/0377-8398(80)90004-3.

Darling, K. F., Kučera, M., Kroon, D., and Wade, Ch. M. (2006) A resolution for the coiling direction paradox in *Neogloboquadrina pachyderma*, *Paleoceanography* 21 (2): PA2011, doi:10.1029/2005PA001189.

Darling, K. F., Kučera, M., Wade, Ch. M., von Langen, P., and Pak, D. (2003) Seasonal distribution of genetic types of planktonic foraminifer morphospecies in the Santa Barbara Channel and its paleoceanographic implications, *Paleoceanography* 18 (2): Article 1032, doi:10.1029/2001PA000723.

Darling, K. F., Thomas, E., Kasemann, S. A., Seears, H. A., Smart, Ch. W., and Wade, Ch. M. (2009) Surviving mass extinction by bridging the benthic/planktic divide, *Proceedings of the National Academy of Sciences of the United States of America* 106 (31): 12629–33, doi:10.1073/pnas.0902827106.

Darling, K. F. and Wade, Ch. M. (2008) The genetic diversity of planktic Foraminifera and the global distribution of ribosomal RNA genotypes, *Marine Micropaleontology* 67: 216–38, doi:10.1016/j.marmicro.2008.01.009.

Darling, K. F., Wade, Ch. M., Kroon, D., and Leigh Brown, A. J. (1997) Planktic foraminiferal molecular evolution and their polyphyletic origins from benthic taxa, *Marine Micropaleontology* 30 (4): 251–66, doi:10.1016/S0377-8398(96)00057-6.

Davis, C. V., Badger, M. P. S., Bown, P. R., and Schmidt, D. N. (2013) The response of calcifying plankton to climate change in the Pliocene, *Biogeosciences* 10 (4): 6131–9, doi:10.5194/bg-10-6131-2013.

De Coster, G., Van Dongen, S., Malaki, P., Muchane, M., Alcántara-Exposito, A., Matheve, H., and Lens, L. (2013) Fluctuating asymmetry and environmental stress: Understanding the role of trait history, *PLOS ONE* 8 (3): e57966, doi:10.1371/journal.pone.0057966.

Debat, V. and David, P. (2001) Mapping phenotypes: Canalization, plasticity and developmental stability, *Trends in Ecology and Evolution* 16 (10): 555–61, doi:10.1016/S0169-5347(01)02266-2.

Debat, V., Milton, C. C., Rutherford, S., Klingenberg, Ch. P., and Hoffmann, A. A. (2006) Hsp90 and the quantitative variation of wing shape in *Drosophila melanogaster*, *Evolution: International Journal of Organic Evolution* 60 (12): 2529–38, doi:10.1554/06-045.1.

Debat, V. and Peronnet, F. (2013) Asymmetric flies: The control of developmental noise in *Drosophila*, *Fly* 7 (2): 70–7, doi:10.4161/fly.23558.

Debenay, J.-P., Della Patrona, L., Herbland, A., and Goguenheim, H. (2009) The impact of easily oxidized material (EOM) on the meiobenthos: Foraminifera abnormalities in shrimp ponds of New Caledonia; implications for environment and paleoenvironment survey, *Marine Pollution Bulletin* 59: 323–35, doi:10.1016/j.marpolbul.2009.08.012.

Doebeli, M. (1996) A quantitative genetic competition model for sympatric speciation, *Journal of Evolutionary Biology* 9 (6): 893–909, doi:10.1046/j.1420-9101.1996.9060893.x.

Dow, R. L. (1977) Test porosity in *Neogloboquadrina pachyderma* in late Miocene to recent, DSDP Site 284 South Pacific, *Palaeogeography, Palaeoclimatology, Palaeoecology* 21 (2): 157–62, doi:10.1016/0031-0182(77)90011-6.

van Eijden, A. J. M. and Ganssen, G. M. (1995) An Oligocene multi-species foraminiferal oxygen and carbon isotope record from ODP Hole 758A (Indian Ocean): Paleoceanographic and paleo-ecologic implications, *Marine Micropaleontology* 25: 47–65, doi:10.1016/0377-8398(94)00028-L.

Emiliani, C. (1971) Depth habitats of growth stages of pelagic Foraminifera, *Science* 173 (4002): 1122–4, doi:10.1126/science.173.4002.1122.

Erez, J. and Honjo, S. (1981) Comparison of isotopic composition of planktonic Foraminifera in plankton tows, sediment traps and sediments, *Palaeogeography, Palaeoclimatology, Palaeoecology* 33 (1–3): 129–56, doi:10.1016/0031-0182(81)90035-3.

Ericson, D. B. (1959) Coiling direction of *Globigerina pachyderma* as a climatic index, *Science* 130 (3369): 219–20, doi:10.1126/science.130.3369.219.

Ericson, D. B., Wollin, G., and Wollin, J. (1955) Coiling direction of *Globorotalia truncatulinoides* in deep-sea cores, *Deep-Sea Research* 2 (2): 153–8, doi:10.1016/0146-6313(55)90018-6.

Fenton, M., Geiselhart, S., Rohling, E. J., and Hemleben, Ch. (2000) Aplanktonic zones in the Red Sea, *Marine Micropaleontology* 40 (3): 277–94, doi:10.1016/S0377-8398(00)00042-6.

Frontalini, F., Buosi, C., Da Pelo, S., Coccioni, R., Cherchi, A., and Bucci, C. (2009) Benthic Foraminifera as bio-indicators of trace element pollution in the heavily contaminated Santa Gilla Lagoon (Cagliari, Italy), *Marine Pollution Bulletin* 58: 858–77, doi:10.1016/j.marpolbul.2009.01.015.

Galilei, G. (1638) *Discorsi e dimostrazioni matematiche intorno à due nuove scienze [Discourses and Mathematical Demonstrations Relating to Two New Sciences]*, (Leiden: Lodewijk Elzevir), http://oll.libertyfund.org/?option=com_staticxt&staticfile=show.php?title=753.

Ganssen, G. M., Peeters, F. J. C., Metcalfe, B., Anand, P., Jung, S. J. A., Kroon, D., and Brummer, G.-J. A. (2011) Quantifying sea surface temperature ranges of the Arabian Sea for the past 20000 years, *Climate of the Past* 7 (4): 1337–49, doi:10.5194/cp-7-1337-2011.

Gast, R. J. and Caron, D. A. (1996) Molecular phylogeny of symbiotic dinoflagellates from planktonic Foraminifera and Radiolaria, *Molecular Biology and Evolution* 13 (9): 1192–7.

Gast, R. J., McDonnell, T. A., and Caron, D. A. (2000) srDNA-based taxonomic affinities of algal symbionts from a planktonic foraminifer and a solitary radiolarian, *Journal of Phycology* 36 (1): 172–7, doi:10.1046/j.1529-8817.2000.99133.x.

Geslin, E., Debenay, J.-P., Duleba, W., and Bonetti, C. (2002) Morphological abnormalities of foraminiferal tests in Brazilian environments: Comparison between polluted and non-polluted areas, *Marine Micropaleontology* 45: 151–68, doi:10.1016/S0377-8398(01)00042-1.

Geslin, E., Stouff, V., Debenay, J.-P., and Lesourd, M. (2000) Environmental variation and foraminiferal test abnormalities, In Martin, R. E. (ed.), *Environmental Micropaleontology: The Application of Microfossils to Environmental Geology*, Topics in Geobiology no. xv, (New York, Boston, Dordrecht, London, Moscow: Kluwer Academic/Plenum Publishers), chap. 9, pp. 191–215.

Graham, J. H., Freeman, D. C., and Emlen, J. M. (1993) Antisymmetry, directional asymmetry, and dynamic morphogenesis, *Genetica* 89 (1–3): 121–37, doi:10.1007/BF02424509.

Grell, K. G. (1973) *Protozoology*, (Berlin, Heidelberg: Springer-Verlag), 554 pp., doi:10.1007/978-3-642-61958-8.

Guiot, J. and de Vernal, A. (2007) Transfer functions: Methods for quantitative paleoceanography based on microfossils, In Hillaire-Marcel, C., de Vernal, A., and Chamley, H. (eds.), *Proxies in Late Cenozoic Paleoceanography*, Developments in Marine Geology no. i, (Amsterdam: Elsevier), chap. 13, pp. 523–63, doi:10.1016/S1572-5480(07)01018-4.

Haenel, P. (1987) Intérêt paléoocéanographique d'*Orbulina universa* d'Orbigny (foraminifère), *Oceanologica Acta* 10 (1): 15–25.

Hallgrímsson, B., Willmore, K., and Hall, B. K. (2002) Canalization, developmental stability, and morphological integration in primate limbs, *American Journal of Physical Anthropology* 119 (Suppl. 35): 131–58, doi:10.1002/ajpa.10182.

Hamilton, Ch. P., Spero, H. J., Bijma, J., and Lea, D. W. (2008) Geochemical investigation of gametogenic calcite addition in the planktonic Foraminifera *Orbulina universa*, *Marine Micropaleontology* 68: 256–67, doi:10.1016/j.marmicro.2008.04.003.

Hammer, Ø. and Harper, D. (2006) *Paleontological Data Analysis*, (Malden, Oxford, Carlton: Blackwell Publishing), 351 pp.

Hannig, L. (2013) 'Die Embryonalentwicklung der Paradiesschnecke *Marisa cornuarietis* (Ampullariidae) unter dem Einfluss von Platin [Embryonal Development of the Rams-Horn Snail *Marisa cornuarietis* (Ampullariidae) under the Influence of Platinum]', Ph.D. thesis, Tübingen: Eberhard–Karls Universität Tübingen, 134 pp., https://publikationen.uni-tuebingen.de/xmlui/handle/10900/50024.

Hansen, Th. F. (2006) The evolution of genetic architecture, *Annual Review of Ecology, Evolution, and Systematics* 37: 123–57, doi:10.1146/annurev.ecolsys.37.091305.110224.

Hart, M. B., Oxford, M. B., and Hudson, W. (2002) The early evolution and palaeobiogeography of Mesozoic planktonic Foraminifera, In Crame, J. A. and Owen, A. W. (eds.), *Palaeobiogeography and Biodiversity Change: The Ordovician and Mesozoic–Cenozoic Radiations*, Special Publications no. cxciv, (London: The Geological Society), pp. 115–25, doi:10.1144/GSL.SP.2002.194.01.09.

Hayden, E. J., Weikert, Ch., and Wagner, A. (2012) Directional selection causes decanalization in a group I ribozyme, *PLOS ONE* 7 (9): e45351, doi:10.1371/journal.pone.0045351.

Healy-Williams, N. (1983/84) Fourier shape analysis of *Globorotalia truncatulinoides* from late Quaternary sediments in the southern Indian Ocean, *Marine Micropaleontology* 8: 1–15, doi:10.1016/0377-8398(83)90002-6.

Healy-Williams, N., Ehrlich, R., and Williams, D. F. (1985) Morphometric and stable isotopic evidence for subpopulations of *Globorotalia truncatulinoides*, *Journal of Foraminiferal Research* 15 (4): 242–53, doi:10.2113/gsjfr.15.4.242.

Healy-Williams, N. and Williams, D. F. (1981) Fourier analysis of test shape of planktonic Foraminifera, *Nature* 289: 485–7, doi:10.1038/289485a0.

Hecht, A. D. (1974) Intraspecific variation in recent populations of *Globigerinoides ruber* and *Globigerinoides trilobus* and their application to paleoenvironmental analysis, *Journal of Paleontology* 48 (6): 1217–34, http://www.jstor.org/stable/1303401.

Hecht, A. D. (1976) An ecologic model for test size variation in recent planktonic Foraminifera: Applications to the fossil record, *Journal of Foraminiferal Research* 6: 295–311, doi:10.2113/gsjfr.6.4.295.

Hemleben, Ch., Becker, T., Bellas, S., Benningsen, G., Casford, J., Cagatay, N., Emeis, K.-Ch., Engelen, B., Ertan, T., Fontanier, Ch., Friedrich, O., Frydas, D., Giunta, S., Hoffelner, H., Jorissen, F., Kahl, G., Kaszemeik, K., Lykousis, V., Meier, S., Nickel, G., Overman, J., Pross, J., Reichel, T., Robert, C., Rohling, E., Ruschmeier, W., Sakinc, M., Schiebel, R., Schmiedl, G., Schubert, K., Schulz, H., Tischnak, J., and Truscheit, T. (2003) *Ostatlantik–Mittelmeer–Schwarzes Meer Part 3: Cruise No. 51, Leg 3 [Eastern Atlantic Ocean–Mediterranean Sea–Black*

*Sea Part 3: Cruise No. 51, Leg 3]*, vol. 3, Meteor Berichte, (Hamburg: Leitstelle Meteor), 57 pp., http://www.dfg-ozean.de/fileadmin/DFG/Berichte/m51-3.pdf.

Hemleben, Ch. and Bijma, J. (1994) Foraminiferal population dynamics and stable carbon isotopes, In Zahn, R., Pedersen, T. F., Kaminski, M. A., and Labeyrie, L. (eds.), *Carbon Cycling in the Glacial Ocean Constraints on the Ocean's Role in Global Change*, vol. 17, NATO ASI Series no. i, (Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong: Springer-Verlag), pp. 145–66.

Hemleben, Ch., Spindler, M., and Anderson, O. R. (1989) *Modern Planktonic Foraminifera*, (New York: Springer-Verlag), 363 pp.

Hemleben, Ch., Spindler, M., Breitinger, I., and Ott, R. (1987) Morphological and physiological responses of *Globigerinoides sacculifer* (Brady) under varying laboratory conditions, *Marine Micropaleontology* 12: 305–24, doi:10.1016/0377-8398(87)90025-9.

Hendrickx, F., Maelfait, J.-P., and Lens, L. (2003) Relationship between fluctuating asymmetry and fitness within and between stressed and unstressed populations of the wolf spider *Pirata piraticus*, *Journal of Evolutionary Biology* 16: 1270–9, doi:10.1046/j.1420-9101.2003.00633.x.

Hesemann, M. (2009) The Foraminifera.eu internet project, In Peryt, D. and Kaminski, M. A. (eds.), *7th Micropalaeontological Workshop Abstracts and Excursion Guide: Mikro-2009, Sw. Katarzyna, Poland, 28–30 September 2009*, Special Publications 15, (Grzybowski Foundation), pp. 33–4, http://gf.tmsoc.org/Documents/Mikro2009/GFSP15.pdf.

Hoffmann, A. A. and Woods, R. (2001) Trait variability and stress: Canalization, developmental stability and the need for a broad approach, *Ecology Letters* 4 (2): 97–101, doi:10.1046/j.1461-0248.2001.00205.x.

Huber, B. T., Bijma, J., and Darling, K. (1997) Cryptic speciation in the living planktonic foraminifer *Globigerinella siphonifera* (d'Orbigny), *Paleobiology* 23 (1): 33–62, http://www.jstor.org/stable/2401156.

Hunt, G. (2007) The relative importance of directional change, random walks, and stasis in the evolution of fossil lineages, *Proceedings of the National Academy of Sciences of the United States of America* 104 (47): 18404–8, doi:10.1073/pnas.0704088104.

Ingleby, B. and Huddleston, M. (2007) Quality control of ocean temperature and salinity profiles — historical and real-time data, *Journal of Marine Systems* 65 (1–4): 158–75, doi:10.1016/j.jmarsys.2005.11.019.

Itou, M., Ono, T., Oba, T., and Noriki, S. (2001) Isotopic composition and morphology of living *Globorotalia scitula*: A new proxy of sub-intermediate ocean carbonate chemistry?, *Marine Micropaleontology* 42: 189–210, doi:10.1016/S0377-8398(01)00015-9.

Jolicoeur, P. (1963) The generalization of the allometry equation, *Biometrics* 19: 497–9.

Kawecki, T. J. (2000) The evolution of genetic canalization under fluctuating selection, *Evolution: International Journal of Organic Evolution* 54 (1): 1–12, doi:10.1111/j.0014-3820.2000.tb00001.x.

Keeling, P., Leander, B. S., and Simpson, A. (2009) *Eukaryotes: Eukaryota, Organisms with Nucleated Cells*, Tree of Life Web Project Protist Diversity Workshop, http://tolweb.org/Eukaryotes/3.

Kennett, J. P. (1968a) *Globorotalia truncatulinoides* as a paleo-oceanographic index, *Science* 159 (3822): 1461–3, doi:10.1126/science.159.3822.1461.

Kennett, J. P. (1968b) Latitudinal variation in *Globigerina pachyderma* (Ehrenberg) in surface sediments of the southwest Pacific Ocean, *Micropaleontology* 14 (3): 305–18, doi:10.2307/1484691.

Kennett, J. P. (1969) Distribution of planktonic Foraminifera in surface sediments south-east of New Zealand, In Brönnimann, P. and Renz, H. H. (eds.), *Proceedings of the First International Conference on Planktonic Microfossils*, vol. 1, (Leiden), pp. 307–22.

Kennett, J. P. (1976) Phenotypic variation in some recent and late Cenozoic planktonic Foraminifera, In Hedley, R. H. and Adams, C. G. (eds.), *Foraminifera*, vol. 2, (New York: Academic Press), pp. 111–70.

Klingenberg, Ch. P. (2003) A developmental perspective on developmental instability: Theory, models and mechanisms, In Polak, M. (ed.), *Developmental Instability: Causes and Consequences*, (New York: Oxford University Press), pp. 14–34.

Klingenberg, Ch. P. (2008) Morphological integration and developmental modularity, *Annual Review of Ecology, Evolution, and Systematics* 39: 115–32, doi:10.1146/annurev.ecolsys.37.091305.110054.

Klingenberg, Ch. P., Duttke, S., Whelan, S., and Kim, M. (2012) Developmental plasticity, morphological variation and evolvability: A multilevel analysis of morphometric integration in the shape of compound leaves, *Journal of Evolutionary Biology* 25 (1): 115–29, doi:10.1111/j.1420-9101.2011.02410.x.

Kremling, K., Lentz, U., Zeitschel, B., Schulz-Bull, D. E., and Duinker, J. C. (1996) New type of time-series sediment trap for the reliable collection of inorganic and organic trace chemical substances, *Review of Scientific Instruments* 67 (12): 4360–3, doi:10.1063/1.1147582.

Kučera, M. (2007) Planktonic Foraminifera as tracers of past oceanic environments, In Hillaire-Marcel, C., de Vernal, A., and Chamley, H. (eds.), *Proxies in Late Cenozoic Paleoceanography*, Developments in Marine Geology no. i, (Amsterdam: Elsevier), chap. 6, pp. 213–62, doi:10.1016/S1572-5480(07)01011-1.

Kučera, M., Weinelt, M., Kiefer, Th., Pflaumann, U., Hayes, A., Weinelt, M., Chen, M.-T., Mix, A. C., Barrows, T. T., Cortijo, E., Duprat, J., Juggins, S., and Waelbroeck, C. (2005) Reconstruction of sea-surface temperatures from assemblages of planktonic Foraminifera: Multi-technique approach based on geographically constrained calibration data sets and its application to glacial Atlantic and Pacific Oceans, *Quaternary Science Reviews* 24: 951–98, doi:10.1016/j.quascirev.2004.07.014.

Kuroyanagi, A. and Kawahata, H. (2004) Vertical distribution of living planktonic Foraminifera in the seas around Japan, *Marine Micropaleontology* 53: 173–96, doi:10.1016/j.marmicro.2004.06.001.

Le Cadre, V. and Debenay, J.-P. (2006) Morphological and cytological responses of *Ammonia* (Foraminifera) to copper contamination: Implication for the use of Foraminifera as bioindicators of pollution, *Environmental Pollution* 143: 304–17, doi:10.1016/j.envpol.2005.11.033.

Lens, L., Van Dongen, S., Kark, S., and Matthysen, E. (2002) Fluctuating asymmetry as an indicator of fitness: Can we bridge the gap between studies?, *Biological Reviews of the Cambridge Philosophical Society* 77: 27–38, doi:10.1017/S1464793101005796.

Leung, B., Forbes, M. R., and Houle, D. (2000) Fluctuating asymmetry as a bioindicator of stress: Comparing efficacy of analyses involving multiple traits, *The American Naturalist* 155 (1): 101–15, doi:10.1086/303298.

Lin, H.-L., Sheu, D. D.-D., Yang, Y., Chou, W.-C., and Hung, G.-W. (2011) Stable isotopes in modern planktonic Foraminifera: Sediment trap and plankton tow results from the South China Sea, *Marine Micropaleontology* 79: 15–23, doi:10.1016/j.marmicro.2010.12.002.

Linnæus, C. (1758) *Systema Naturæ*, 10th ed., vol. 1, (Stockholm: Laurentius Salvius), 173 pp.

Lohmann, G. P. (1995) A model for variation in the chemistry of planktonic Foraminifera due to secondary calcification selective dissolution, *Paleoceanography* 10 (3): 445–57, doi:10.1029/95PA00059.

Lohmann, G. P. and Malmgren, B. A. (1983) Equatorward migration of *Globorotalia truncatulinoides* ecophenotypes through the Late Pleistocene: Gradual evolution or ocean change?, *Paleobiology* 9 (4): 414–21, http://www.jstor.org/stable/2400583.

Lombard, F., Labeyrie, L., Michel, E., Bopp, L., Cortijo, E., Retailleau, S., Howa, H., and Jorissen, F. (2011) Modelling planktic foraminifer growth and distribution using an ecophysiological multi-species approach, *Biogeosciences* 8: 853–73, doi:10.5194/bg-8-853-2011.

Ludwig, D. (1999) Is it meaningful to estimate a probability of extinction?, *Ecology* 80: 298–310, doi:10.1890/0012-9658(1999)080[0298:IIMTEA]2.0.CO;2.

MacLeod, N. (1990) Effects of late Eocene impacts on planktic Foraminifera, In Sharpton, V. L. and Ward, P. D. (eds.), *Global Catastrophes in Earth History: An Interdisciplinary Conference on Impacts, Volcanism, and Mass Mortality*, Special Papers no. ccxlvii, (Boulder: The Geological Society of America), pp. 595–606.

MacLeod, N., Keller, G., and Kitchell, J. A. (1990) Progenesis in Late Eocene populations of *Subbotina linaperta* (Foraminifera) from the western Atlantic, *Marine Micropaleontology* 16: 219–40, doi:10.1016/0377-8398(90)90005-7.

MacLeod, N., Ortiz, N., Fefferman, N., Clyde, W., Schulter, Ch., and MacLean, J. (2000) Phenotypic response of Foraminifera to episodes of global environmental change, In Culver, S. J. and Rawson, P. F. (eds.), *Biotic Response to Global Change: The Last 145 Million Years*, (Cambridge: Cambridge University Press), chap. 5, pp. 51–78.

Malmgren, B. A. (1984) Analysis of the environmental influence on the morphology of *Ammonia beccarii* (Linné) in southern European salinas, *Geobios* 17 (6): 737–46.

Malmgren, B. A. and Healy-Williams, N. (1978) Variation in test diameter of *Orbulina universa* in the paleoclimatology of the late Quaternary of the Gulf of Mexico, *Palaeogeography, Palaeoclimatology, Palaeoecology* 25: 235–40, doi:10.1016/0031-0182(78)90038-X.

Malmgren, B. A. and Kennett, J. P. (1976) Biometric analysis of phenotypic variation in recent *Globigerina bulloides* d'Orbigny in the southern Indian Ocean, *Marine Micropaleontology* 1: 3–25, doi:10.1016/0377-8398(76)90003-7.

Malmgren, B. A. and Kennett, J. P. (1978a) Late Quaternary paleoclimatic applications of mean size variations in *Globigerina bulloides* d'Orbigny in the southern Indian Ocean, *Journal of Paleontology* 52 (6): 1195–207.

Malmgren, B. A. and Kennett, J. P. (1978b) Test size variation in *Globigerina bulloides* in response to Quaternary palaeoceanographic changes, *Nature* 275: 123–4, doi:10.1038/275123a0.

Manno, C., Morata, N., and Bellerby, R. (2012) Effect of ocean acidification and temperature increase on the planktonic foraminifer *Neogloboquadrina pachyderma* (sinistral), *Polar Biology* 35: 1311–9, doi:10.1007/s00300-012-1174-7.

Marshall, B. J., Thunell, R. C., Henehan, M. H., Astor, Y., and Wejnert, K. R. (2013) Planktonic foraminiferal area density as a proxy for carbonate ion concentration: A calibration study using the Cariaco Basin Ocean Time Series, *Paleoceanography* 28: 1–14, doi:10.1002/palo.20034.

Milton, C. C., Huynh, B., Batterham, Ph., Rutherford, S. L., and Hoffmann, A. A. (2003) Quantitative trait symmetry independent of Hsp90 buffering: Distinct modes of genetic canalization and developmental stability, *Proceedings of the National Academy of Sciences of the United States of America* 100 (23): 13396–401, doi:10.1073/pnas.1835613100.

de Moel, H., Ganssen, G. M., Peeters, F. J. C., Jung, S. J. A., Kroon, D., Brummer, G.-J. A., and Zeebe, R. E. (2009) Planktic foraminiferal shell thinning in the Arabian Sea due to anthropogenic ocean acidification?, *Biogeosciences* 6: 1917–25, doi:10.5194/bg-6-1917-2009.

Moller, T., Schulz, H., and Kučera, M. (2013) The effect of sea surface properties on shell morphology and size of the planktonic foraminifer *Neogloboquadrina pachyderma* in the North Atlantic, *Palaeogeography, Palaeoclimatology, Palaeoecology* 391: 34–48, doi:10.1016/j.palaeo.2011.08.014.

Morard, R., Quillévéré, F., Douady, Ch. J., de Vargas, C., de Garidel-Thoron, Th., and Escarguel, G. (2011) Worldwide genotyping in the planktonic foraminifer *Globoconella inflata*: Implications for life history and paleoceanography, *PLOS ONE* 6 (10): e26665, doi:10.1371/journal.pone.0026665.

Morard, R., Quillévéré, F., Escarguel, G., Ujiie, Y., de Garidel-Thoron, Th., Norris, R. D., and de Vargas, C. (2009) Morphological recognition of cryptic species in the planktonic foraminifer *Orbulina universa*, *Marine Micropaleontology* 71: 148–65, doi:10.1016/j.marmicro.2009.03.001.

Naidu, P. D. and Malmgren, B. A. (1995) Monsoon upwelling effects on test size of some planktonic foraminiferal species from the Oman Margin, Arabian Sea, *Paleoceanography* 10 (1): 117–22, doi:10.1029/94PA02682.

Naidu, P. D. and Malmgren, B. A. (1996) Relationship between late Quaternary upwelling history and coiling properties of *Neogloboquadrina pachyderma* and *Globigerina bulloides* in the Arabian Sea, *Journal of Foraminiferal Research* 26 (1): 64–70, doi:10.2113/gsjfr.26.1.64.

Naik, S. S., Godad, S. P., Naidu, P. D., and Ramaswamy, V. (2013) A comparison of *Globigerinoides ruber* calcification between upwelling and non-upwelling regions in the Arabian Sea, *Journal of Earth System Science* 122 (4): 1153–9, doi:10.1007/s12040-013-0330-y.

Nellen, W., Bettac, W., Roether, W., Schnack, D., Thiel, H., Weikert, H., and Zeitschel, B. (1996) MINDIK *Reise Nr. 5 [*MINDIK *Cruise No. 5]*, Meteor Berichte, (Hamburg: Leitstelle Meteor), 179 pp., http://www.dfg-ozean.de/fileadmin/DFG/Berichte/M5b_Meteor_96-2.pdf.

Niebler, H.-S., Hubberten, H.-W., and Gersonde, R. (1999) Oxygen isotope values of planktic Foraminifera: A tool for the reconstruction of surface stratification, In Fischer, G. and Wefer, G. (eds.), *Use of Proxies in Paleoceanography: Examples from the South Atlantic*, (Berlin, Heidelberg: Springer-Verlag), pp. 165–89.

Norris, R. D. (2000) Pelagic species diversity, biogeography, and evolution, *Paleobiology* 26 (4): 236–58, doi:10.1666/0094-8373(2000)26[236:PSDBAE]2.0.CO;2.

d'Orbigny, A. D. (1826) Tableau méthodique de la classe des Céphalopodes, *Annales des Sciences Naturelles comprenant la physiologie animale et végétale, l'anatomie comparée des deux règnes, la zoologie, la botanique, la minéralogie et la géologie* 1 (7): 1–277.

d'Orbigny, A. D. (1839) Foraminifères, In de la Sagra, R. (ed.), *Histoire physique et naturelle de l'Ile de Cuba*, (Paris: A. Bertrand), p. 82.

Ortiz, J. D., Mix, A. C., and Collier, R. W. (1995) Environmental control of living symbiotic and asymbiotic Foraminifera of the California Current, *Paleoceanography* 10 (6): 987–1009, doi:10.1029/95PA02088.

Pawlowski, J. (2000) Introduction to the molecular systematics of Foraminifera, *Micropaleontology* 46: 1–12, http://www.jstor.org/stable/1486176.

Pélabon, Ch., Hansen, Th. F., Carter, A. J. R., and Houle, D. (2010) Evolution of variation and variability under fluctuating, stabilizing, and disruptive selection, *Evolution: International Journal of Organic Evolution* 64 (7): 1912–25, doi:10.1111/j.1558-5646.2010.00979.x.

Philippi, T. and Seger, J. (1989) Hedging one's evolutionary bets, revisited, *Trends in Ecology and Evolution* 4 (2): 41–4, doi:10.1016/0169-5347(89)90138-9.

Pujol, C. and Vergnaud Grazzini, C. (1995) Distribution patterns of live planktic foraminifers as related to regional hydrography and productive systems of the Mediterranean Sea, *Marine Micropaleontology* 25: 187–217, doi:10.1016/0377-8398(95)00002-I.

Ravelo, A. Ch. and Hillaire-Marcel, C. (2007) The use of oxygen and carbon isotopes of Foraminifera in paleoceanography, In Hillaire-Marcel, C., de Vernal, A., and Chamley, H. (eds.), *Proxies in Late Cenozoic Paleoceanography*, Developments in Marine Geology no. i, (Amsterdam: Elsevier), chap. 18, pp. 735–64, doi:10.1016/S1572-5480(07)01023-8.

Renaud, S. and Schmidt, D. N. (2003) Habitat tracking as a response of the planktic foraminifer *Globorotalia truncatulinoides* to environmental fluctuations during the last 140 kyr, *Marine Micropaleontology* 49 (1–2): 97–122, doi:10.1016/S0377-8398(03)00031-8.

Robbins, L. L. (1988) Environmental significance of morphologic variability in open-ocean versus ocean-margin assemblages of *Orbulina universa*, *Journal of Foraminiferal Research* 18 (4): 326–33, doi:10.2113/gsjfr.18.4.326.

Rohlf, F. J. and Marcus, L. F. (1993) A revolution in morphometrics, *Trends in Ecology and Evolution* 8 (4): 129–32, doi:10.1016/0169-5347(93)90024-J.

Rohling, E. J., Cane, T. R., Cooke, S., Sprovieri, M., Bouloubassi, I., Emeis, K.-Ch., Schiebel, R., Kroon, D., Jorissen, F. J., Lorre, A., and Kemp, A. E. S. (2002) African monsoon variability during the previous interglacial maximum, *Earth and Planetary Science Letters* 202: 61–75, doi:10.1016/S0012-821X(02)00775-6.

Rohling, E. J., de Rijk, S., Myers, P. G., and Haines, K. (2000) Palaeoceanography and numerical modelling: The Mediterranean Sea at times of sapropel formation, In Hart, M. B. (ed.), *Climates: Past and Present*, Special Publications no. clxxxi, (London: The Geological Society), pp. 135–49, doi:10.1144/GSL.SP.2000.181.01.13.

Rossignol-Strick, M., Nesteroff, W., Olive, Ph., and Vergnaud Grazzini, C. (1982) After the deluge: Mediterranean stagnation and sapropel formation, *Nature* 295: 105–10, doi:10.1038/295105a0.

Rutherford, S., D'Hont, S., and Prell, W. (1999) Environmental controls on the geographic distribution of zooplankton diversity, *Nature* 400: 749–53, doi:10.1038/23449.

Sánchez-Chardi, A., García-Pando, M., and López-Fuster, M. J. (2013) Chronic exposure to environmental stressors induces fluctuating asymmetry in shrews inhabiting protected Mediterranean sites, *Chemosphere* 93 (6): 916–23, doi:10.1016/j.chemosphere.2013.05.056.

Santos, M., Iriarte, P. F., and Céspedes, W. (2005) Genetics and geometry of canalization and developmental stability in *Drosophila subobscura*, *BMC Evolutionary Biology* 5: Article 7, doi:10.1186/1471-2148-5-7.

Schiebel, R. (2002) Planktic foraminiferal sedimentation and the marine calcite budget, *Global Biogeochemical Cycles* 16 (4): Article 3, doi:10.1029/2001GB001459.

Schiebel, R. and Hemleben, Ch. (2005) Modern planktic Foraminifera, *Paläontologische Zeitschrift* 79 (1): 135–48, doi:10.1007/BF03021758.

Schiebel, R., Waniek, J., Zeltner, A., and Alves, M. (2002) Impact of the Azores Front on the distribution of planktic foraminifers, shelled gastropods, and coccolithophorids, *Deep-Sea Research, Part II: Topical Studies in Oceanography* 49: 4035–50, doi:10.1016/S0967-0645(02)00141-8.

Schmalhauzen, I. I. (1949) *Factors of Evolution: The Theory of Stabilizing Selection*, (Madison: Blakiston Company), 327 pp.

Schmidt, D. N., Lazarus, D., Young, J. R., and Kučera, M. (2006) Biogeography and evolution of body size in marine plankton, *Earth-Science Reviews* 78: 239–66, doi:10.1016/j.earscirev.2006.05.004.

Schmidt, D. N., Renaud, S., and Bollmann, J. (2003) Response of planktic foraminiferal size to late Quaternary climate change, *Paleoceanography* 18 (2): Article 1039, doi:10.1029/2002PA000831.

Schmidt, D. N., Renaud, S., Bollmann, J., Schiebel, R., and Thierstein, H. R. (2004) Size distribution of Holocene planktic foraminifer assemblages: Biogeography, ecology and adaptation, *Marine Micropaleontology* 50: 319–38, doi:10.1016/S0377-8398(03)00098-7.

Sharifi, A. R., Croudace, I. W., and Austin, R. L. (1991) Benthic foraminiferids as pollution indicators in Southampton water, southern England, U.K., *Journal of Micropalaeontology* 10 (1): 109–13, doi:10.1144/jm.10.1.109.

Siccha, M., Trommer, G., Schulz, H., Hemleben, Ch., and Kučera, M. (2009) Factors controlling the distribution of planktonic Foraminifera in the Red Sea and implications for the development of transfer functions, *Marine Micropaleontology* 72: 146–56, doi:10.1016/j.marmicro.2009.04.002.

Siegal, M. L. and Bergman, A. (2002) Waddington's canalization revisited: Developmental stability and evolution, *Proceedings of the National Academy of Sciences of the United States of America* 99 (16): 10528–32, doi:10.1073/pnas.102303999.

Sierra, R., Matz, M. V., Aglyamova, G., Pillet, L., Decelle, J., Not, F., de Vargas, C., and Pawlowski, J. (2013) Deep relationships of Rhizaria revealed by phylogenomics: A farewell to Haeckel's Radiolaria, *Molecular Phylogenetics and Evolution* 67 (1): 53–9, doi:10.1016/j.ympev.2012.12.011.

Slatkin, M. (1974) Hedging one's evolutionary bets, *Nature* 250 (5469): 704–5, doi:10.1038/250704b0.

Smith, V., Sausville, E. A., Camalier, R. F., Fiebig, H.-H., and Burger, A. M. (2005) Comparison of 17-dimethylaminoethylamino-17-demethoxy-geldanamycin (17DMAG) and 17-allylamino-17-demethoxygeldanamycin (17AAG) *in vitro*: Effects on Hsp90 and client proteins in melanoma models, *Cancer Chemotherapy and Pharmacology* 56: 126–37, doi:10.1007/s00280-004-0947-2.

Spero, H. J. (1988) Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*, *Marine Biology* 99 (1): 9–20, doi:10.1007/BF00644972.

Spero, H. J., Bijma, J., Lea, D. W., and Bemis, B. E. (1997) Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes, *Nature* 390: 497–500, doi:10.1038/37333.

Spero, H. J. and Lea, D. W. (1993) Intraspecific stable isotope variability in the planktic Foraminifera *Globigerinoides sacculifer*: Results from laboratory experiments, *Marine Micropaleontology* 22: 221–34, doi:10.1016/0377-8398(93)90045-Y.

Spindler, M., Hemleben, Ch., Bayer, U., Bé, A. W. H., and Anderson, O. R. (1979) Lunar periodicity of reproduction in the planktonic foraminifer *Hastigerina pelagica*, *Marine Ecology Progress Series* 1: 61–4.

Steinke, S., Chiu, H.-Y., Yu, P.-S., Shen, C.-C., Löwemark, L., Mii, H.-S., and Chen, M.-T. (2005) Mg/ca ratios of two *Globigerinoides ruber* (white) morphotypes: Implications for reconstructing past tropical/subtropical surface water conditions, *Geochemistry, Geophysics, Geosystems* 6 (11): Q11005, doi:10.1029/2005GC000926.

Storz, D., Schulz, H., Waniek, J. J., Schulz-Bull, D. E., and Kučera, M. (2009) Seasonal and interannual variability of the planktic foraminiferal flux in the vicinity of the Azores

Current, *Deep-Sea Research, Part I: Oceanographic Research Papers* 56: 107–24, doi:10.1016/j.dsr.2008.08.009.

Sundberg, P. (1989) Shape and size-constrained principal component analysis, *Systematic Zoology* 38 (2): 166–8, doi:10.2307/2992385.

Takahashi, K. H., Daborn, Ph. J., Hoffmann, A. A., and Takano-Shimizu, T. (2011) Environmental stress-dependent effects of deletions encompassing *Hsp70Ba* on canalization and quantitative trait asymmetry in *Drosophila melanogaster*, *PLOS ONE* 6 (4): e17295, doi:10.1371/journal.pone.0017295.

Takahashi, K. H., Rako, L., Takano-Shimizu, T., Hoffmann, A. A., and Lee, S. F. (2010) Effects of small *Hsp* genes on developmental stability and microenvironmental canalization, *BMC Evolutionary Biology* 10: Article 284, doi:10.1186/1471-2148-10-284.

Takayanagi, Y., Niitsuma, N., and Sakai, T. (1968) Wall microstructure of *Globorotalia truncatulinoides* (d'Orbigny), *The Science Reports of the Tokohu University* 31: 1477–500.

Telford, R. J. and Kučera, M. (2013) Mismatch between the depth habitat of planktonic Foraminifera and the calibration depth of SST transfer functions may bias reconstructions, *Climate of the Past* 9 (2): 859–70, doi:10.5194/cp-9-859-2013.

Thirumalai, K., Richey, J. N., Quinn, T. M., and Poore, R. Z. (2014) *Globigerinoides ruber* morphotypes in the Gulf of Mexico: A test of null hypothesis, *Scientific Reports* 4: Article 6018, doi:10.1038/srep06018.

Van Valen, L. (1965) Morphological variation and width of ecological niche, *The American Naturalist* 99 (908): 377–90, http://www.jstor.org/stable/2459179.

de Vargas, C., Norris, R., Zaninetti, L., Gibb, S. W., and Pawlowski, J. (1999) Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces, *Proceedings of the National Academy of Sciences of the United States of America* 96: 2864–8, doi:10.1073/pnas.96.6.2864.

de Vargas, C., Renaud, S., Hilbrecht, H., and Pawlowski, J. (2001) Pleistocene adaptive radiation in *Globorotalia truncatulinoides*: Genetic, morphologic, and environmental evidence, *Paleobiology* 27 (1): 104–25, doi:10.1666/0094-8373(2001)027<0104:PARIGT>2.0.CO;2.

Vasicek, M. (1953) Zmeny Vzájemného Pomeru Levotocivych a Pravotocivych Jedincu Foraminifery *Globorotalia scitula* (Brady) a Jejich Vyuzií ve Stratigrafii [Change of the proportion of left- and right-coiling individuals of the foraminifer *Globorotalia scitula* (Brady) and their use in stratigraphy], *Geologický Sborník* 20: 1–76.

de Villiers, S. (2004) Optimum growth conditions as opposed to calcite saturation as a control on the calcification rate and shell-weight of marine Foraminifera, *Marine Biology* 144: 45–9, doi:10.1007/s00227-003-1183-8.

Waddington, C. H. (1942) Canalization of development and the inheritance of aquired characters, *Nature* 150 (3811): 563–5, doi:10.1038/150563a0.

Wagner, G. P. (1996) Homologues, natural kinds and the evolution of modularity, *American Zoologist* 36 (1): 36–43, http://www.jstor.org/stable/3883984.

Wagner, G. P. and Altenberg, L. (1996) Perspective: Complex adaptations and the evolution of evolvability, *Evolution: International Journal of Organic Evolution* 50 (3): 967–76, doi:10.2307/2410639.

Wagner, G. P., Booth, G., and Homayoun, B.-C. (1997) A population genetic theory of canalization, *Evolution: International Journal of Organic Evolution* 51 (2): 329–47, doi:10.2307/2411105.

Wang, L. (2000) Isotopic signals in two morphotypes of *Globigerinoides ruber* (white) from the South China Sea: Implications for monsoon climate change during the last glacial cycle, *Palaeogeography, Palaeoclimatology, Palaeoecology* 161 (3–4): 381–94, doi:10.1016/S0031-0182(00)00094-8.

Weiner, A. K. M., Weinkauf, M. F. G., Kurasawa, A., Darling, K. F., and Kučera, M. (2015) Genetic and morphometric evidence for parallel evolution of the *Globigerinella calida* morphotype, *Marine Micropaleontology* 114: 19–35, doi:10.1016/j.marmicro.2014.10.003.

Weiner, A. K. M. (2014) 'Genetic Diversity, Biogeography and the Morpho–Genetic Relationship in Extant Planktonic Foraminifera', Ph.D. thesis, Bremen: Universität Bremen, 143 pp., `http://elib.suub.uni-bremen.de/edocs/00103933-1.pdf`.

Weiner, A., Aurahs, R., Kurasawa, A., Kitazato, H., and Kučera, M. (2012) Vertical niche partitioning between cryptic sibling species of a cosmopolitan marine planktonic protist, *Molecular Ecology* 21 (16): 4063–73, doi:10.1111/j.1365-294X.2012.05686.x.

Williams, D. F., Thunell, R. C., and Kennett, J. P. (1978) Periodic freshwater flooding and stagnation of the Eastern Mediterranean Sea during the late Quaternary, *Science* 201 (4352): 252–4, doi:10.1126/science.201.4352.252.

Willmore, K. E., Klingenberg, Ch. P., and Hallgrímsson, B. (2005) The relationship between fluctuating asymmetry and environmental variance in rhesus macaque skulls, *Evolution: International Journal of Organic Evolution* 59 (4): 898–909, doi:10.1111/j.0014-3820.2005.tb01763.x.

Workgroup Mikropalaeontology–Palaeoceanography (2013) *Foraminifera Image Database*, MARUM, Universität Bremen, `http://foraminiferaimagedatabase.marum.de/gallery2/main.php`.

Yezerinac, S. M., Lougheed, S. C., and Handford, P. (1992) Measurement error and morphometric studies: Statistical power and observer experience, *Systematic Biology* 41 (4): 471–82, doi:10.1093/sysbio/41.4.471.

Zelditch, M. L., Swiderski, D. L., and Sheets, H. D. (2012) *Geometric Morphometrics for Biologists: A Primer*, 2nd ed., (London, Waltham, San Diego: Academic Press), 478 pp., `http://booksite.elsevier.com/9780123869036/index.php`.

# Part II

# Manuscripts

# Chapter 4

**Manuscript 1**: Weinkauf, M. F. G., Moller, T., Koch, M. C., and Kučera, M. (2013) Calcification intensity in planktonic Foraminifera reflects ambient conditions irrespective of environmental stress, *Biogeosciences* 10 (10): 6639–55, doi:10.5194/bg-10-6639-2013

## Abstract

Planktonic Foraminifera are important marine calcifiers, and the ongoing change in the oceanic carbon system makes it essential to understand the influence of environmental factors on the biomineralization of their shells. The amount of calcite deposited by planktonic Foraminifera during calcification has been hypothesized to reflect a range of environmental factors. However, it has never been assessed whether their calcification only passively responds to the conditions of the ambient seawater or whether it reflects changes in resource allocation due to physiological stress. To disentangle these two end-member scenarios, an experiment is required where the two processes are separated. A natural analogue to such an experiment occurred during the deposition of the Mediterranean sapropels, where large changes in surface water composition and stratification at the onset of the sapropel deposition were decoupled from local extinctions of planktonic Foraminifera species. We took advantage of this natural experiment and investigated the reaction of calcification intensity, expressed as mean area density (MAD), of four species of planktonic Foraminifera to changing conditions during the onset of Sapropel S5 (126–121 ka) in a sediment core from the Levantine Basin. We observed a significant relationship between MAD and surface water properties, as reflected by stable isotopes in the calcite of Foraminifera shells, but we failed to observe any reaction of calcification intensity on ecological stress during times of decreasing abundance culminating in local extinction. The reaction of calcification intensity to surface water perturbation at the onset of the sapropel was observed only in surface-dwelling species, but all species calcified more strongly prior to the sapropel deposition and less strongly within the sapropel than at similar conditions during the present-day. These results indicate that the high-salinity environment of the glacial Mediterranean Sea prior to sapropel deposition induced a more intense calcification, whereas the freshwater injection to the surface waters associated with sapropel deposition inhibited calcification. The results are robust to changes in carbonate preservation and collectively imply that changes in normalized shell weight in planktonic Foraminifera should reflect mainly abiotic forcing.

Biogeosciences, 10, 6639–6655, 2013
www.biogeosciences.net/10/6639/2013/
doi:10.5194/bg-10-6639-2013


Biogeosciences Open Access

# Calcification intensity in planktonic Foraminifera reflects ambient conditions irrespective of environmental stress

**M. F. G. Weinkauf[1,2], T. Moller[1], M. C. Koch[3], and M. Kučera[2]**

[1]Eberhard Karls Universität, Mathematisch–Naturwissenschaftliche Fakultät, Fachbereich Geowissenschaften, Hölderlinstraße 12, 72074 Tübingen, Germany
[2]MARUM, Leobener Straße, 28359 Bremen, Germany
[3]Johann Wolfgang Goethe-Universität, Institut für Geowissenschaften, Facheinheit Paläontologie, Altenhöferallee 1, 60438 Frankfurt am Main, Germany

*Correspondence to:* M. F. G. Weinkauf (mweinkauf@marum.de)

Received: 18 June 2013 – Published in Biogeosciences Discuss.: 8 July 2013
Revised: 6 September 2013 – Accepted: 16 September 2013 – Published: 23 October 2013

**Abstract.** Planktonic Foraminifera are important marine calcifiers, and the ongoing change in the oceanic carbon system makes it essential to understand the influence of environmental factors on the biomineralization of their shells. The amount of calcite deposited by planktonic Foraminifera during calcification has been hypothesized to reflect a range of environmental factors. However, it has never been assessed whether their calcification only passively responds to the conditions of the ambient seawater or whether it reflects changes in resource allocation due to physiological stress. To disentangle these two end-member scenarios, an experiment is required where the two processes are separated. A natural analogue to such an experiment occurred during the deposition of the Mediterranean sapropels, where large changes in surface water composition and stratification at the onset of the sapropel deposition were decoupled from local extinctions of planktonic Foraminifera species. We took advantage of this natural experiment and investigated the reaction of calcification intensity, expressed as mean area density (MAD), of four species of planktonic Foraminifera to changing conditions during the onset of Sapropel S5 (126–121 ka) in a sediment core from the Levantine Basin. We observed a significant relationship between MAD and surface water properties, as reflected by stable isotopes in the calcite of Foraminifera shells, but we failed to observe any reaction of calcification intensity on ecological stress during times of decreasing abundance culminating in local extinction. The reaction of calcification intensity to surface water perturbation at the onset of the sapropel was observed only in surface-dwelling species, but all species calcified more strongly prior to the sapropel deposition and less strongly within the sapropel than at similar conditions during the present-day. These results indicate that the high-salinity environment of the glacial Mediterranean Sea prior to sapropel deposition induced a more intense calcification, whereas the freshwater injection to the surface waters associated with sapropel deposition inhibited calcification. The results are robust to changes in carbonate preservation and collectively imply that changes in normalized shell weight in planktonic Foraminifera should reflect mainly abiotic forcing.

## 1 Introduction

The amount of calcite present in a planktonic foraminifer shell at a certain time in relation to its size, hereafter referred to as calcification intensity, has been suggested to reflect various physical and chemical properties of the ambient seawater that affect the inorganic precipitation of calcite. In contrast to calcification rate, calcification intensity is here used as a measure of calcification independent of the time over which the calcification took place. Decreased shell weight of Foraminifera has been interpreted as resulting from ocean acidification and decreased $CO_3^{2-}$ content of the seawater (Lohmann, 1995; Broecker and Clark, 2001). Therefore, in theory, a reaction of the calcification intensity of Foraminifera on ocean acidification resulting from

anthropogenic atmospheric $CO_2$, could severely influence the oceanic carbon cycle.

On geological timescales a relationship between Foraminifera calcification and the ocean carbonate system has been reported in *Globigerina bulloides* from a sediment core record in the North Atlantic by Barker and Elderfield (2002). The existence of a relationship between carbonate ion concentration and calcification intensity has been confirmed in laboratory culturing studies (Bijma et al., 1999; Lombard et al., 2010). Similarly, observations of shell thinning of planktonic Foraminifera in the Arabian Sea and Southern Ocean have been interpreted as a reaction of these organisms to anthropogenic carbon sequestration in the ocean (de Moel et al., 2009; Moy et al., 2009). However, a subsequent study of plankton material from the Arabian Sea by Beer et al. (2010b) revealed that the relationship between calcification intensity and carbonate ion concentration is not straightforward and may be species-specific, whereas Aldridge et al. (2012) identified phosphate concentration as the strongest determinant of calcification intensity in *G. bulloides* from North Atlantic plankton samples. Marshall et al. (2013) suggested the $CO_3^{2-}$ content of the seawater to be the main influential factor on the calcification intensity of *Globigerinoides ruber* and *Globigerinoides sacculifer* on the basis of trap samples from the Cariaco Basin (Venezuela). However, since temperature was used to calculate the $CO_3^{2-}$ values, the two variables were not independent. For this reason, the authors could not exclude the possibility that ambient temperature played an important role in mediating this relationship. All three studies provided evidence against the hypothesis by de Villiers (2004), that calcification intensity reflects optimum growth conditions, whereas Manno et al. (2012) showed that ambient temperature modulates the effect of changes in carbonate chemistry on calcification in Arctic *Neogloboquadrina pachyderma*.

Fundamentally, factors which are likely to control calcification intensity in planktonic Foraminifera depend on the degree to which the biomineralization process is coupled to physiological processes in the cell. Studies searching for candidate environmental factors affecting calcification assume that the biomineralization mimics inorganic precipitation. However, it is possible that biomineralization in Foraminifera could participate in a trade-off in the allocation of resources between biomass and biomineral. The existence of such trade-off is implied by the hypothesis of de Villiers (2004), which suggests that populations inhabiting environments, where all environmental parameters are close to the optimum of a species, are characterized not only by peak-productivity but also by highest calcification intensities in that species. This is consistent with the observation that size in planktonic Foraminifera reflects optimum growth conditions (Schmidt et al., 2004). Considering the seemingly contradictory results of existing studies on calcification intensity in planktonic Foraminifera, it appears that the process should be considered at a more fundamental level. Specifically, it remains to be established whether calcification intensity simply tracks the conditions of the ambient seawater or whether it reflects a physiological stress reaction of the organism at an appropriate ecological timescale.

Therefore, prior to further work attempting to isolate abiotic factors responsible for differences in calcification intensity in planktonic Foraminifera, the effect of environmental stress on this process has to be characterized. Environmental stress is here defined as the sum of physical, chemical, and biological factors influencing the productivity of a species. Provided growth and calcification are linked by a trade-off in resource allocation, then if environmental stress affects the productivity of a species, it could conceivably play a role for its calcification. Whereas it is difficult to disentangle individual aspects of environmental stress, its net result is easily quantifiable in terms of changes in the biomass of the studied species or population. This quantity is indirectly preserved in the fossil record, making it possible, in principle, to quantify how fossil populations were affected by stress. What is more, unlike the present-day situation, the fossil record allows investigating the effects of environmental stress resulting in a range of a priori known outcomes for the stressed population, including its total demise.

The amount of calcite preserved in fossil Foraminifera can be strongly influenced by post-mortem diagenetic processes. In order to circumvent this complication, the optimal setting to study calcification in the fossil record should be such where oversaturation of the entire water column with respect to carbonate can be demonstrated throughout the target time interval. The Eastern Mediterranean Sea is separated from the Western Mediterranean by the Sicilian Sill and Malta Sill (Wüst, 1961). Due to high evaporation rates in summer, surface ocean salinities can reach values larger than 39 ‰ in the eastern Levantine Basin (Wüst, 1961), making the water column highly oversaturated with respect to calcite (Schneider et al., 2007).

In addition, the Eastern Mediterranean is strongly influenced both by changes in monsoon intensity that alter freshwater input via the Nile, and changes in mid/high latitude climate patterns. Due to its small size and high sensitivity to hydrological processes, the basin amplifies environmental response to climate change (Rohling et al., 2002, 2009), and therefore offers an excellent opportunity to study the reaction of indigenous marine organisms on stress.

Here we take advantage of the unique environmental setting of the Eastern Mediterranean to study the response of calcification intensity in four species of planktonic Foraminifera to the environmental perturbation that led to the deposition of Sapropel S5 (Rohling et al., 2002). The environmental change associated with this perturbation induced a sequence of local extinctions and re-colonizations by planktonic Foraminifera species that can be tracked throughout the whole Eastern Mediterranean (Cane et al., 2002) (Fig. 1). The sapropel deposition reflects an abrupt environmental change leading to enhanced surface stratification and

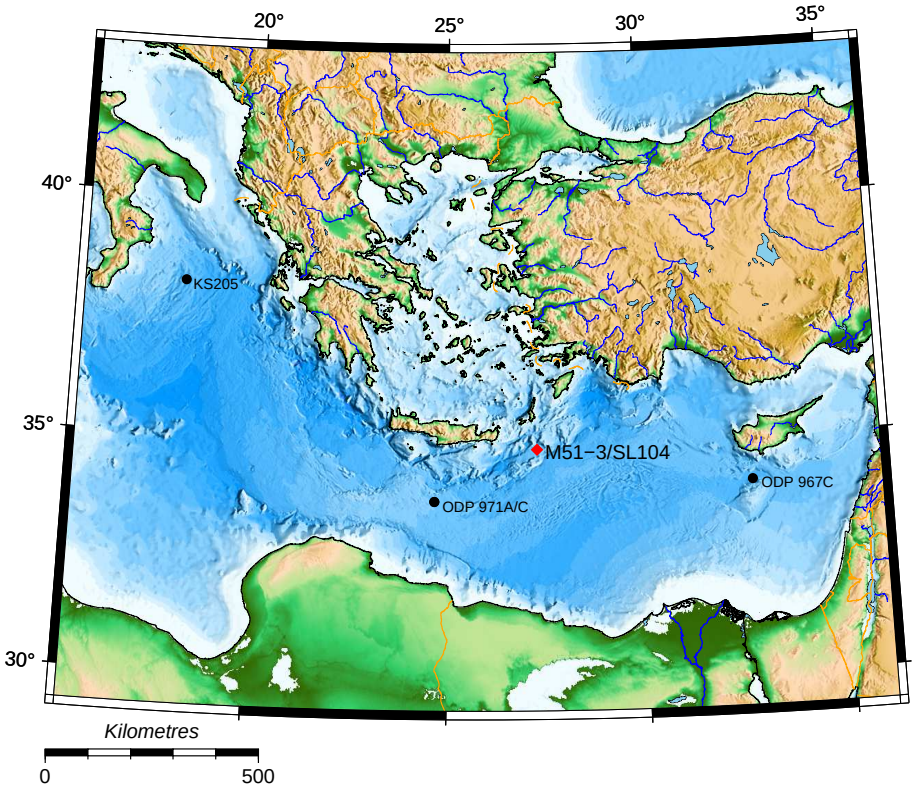



**Fig. 1.** Position of core M51-3/SL104 (red) in the Pliny Trench about 100 km east-southeast of Crete, as well as the position of the four other main cores (black), where the local extinction sequence of planktonic foraminifer species across Sapropel S5 has been established (Cane et al., 2002).

stagnation of the water column (Rossignol-Strick et al., 1982; Rossignol-Strick, 1983; Myers et al., 1998; Rohling et al., 2000). It is reflected in a drop in oxygen and carbon stable isotope values of planktonic Foraminifera at the onset of sapropel deposition, recording enhanced freshwater discharge from Africa (Williams et al., 1978).

Our hypothesis is that there are two possible modes of reaction of Foraminifera calcification intensity to the events surrounding the onset of Sapropel S5 deposition: (a) long-term ecological-scale reactions, due to the persistent environmental change associated with the onset of the sapropel, and (b) short-term physiological-scale effects, associated with the terminal environmental stress leading to local extinction of the species. Point (a) can be further divided into the hypotheses that there is either an influence (a1) of the environmental change itself (abiotic) or (a2) of the environmental stress associated with such a change (biotic) on the calcification intensity of planktonic Foraminifera.

Using palaeontological data extracted from the sediment record, instead of samples taken in recent environments or laboratory cultures, allows us to observe the reaction of natural communities that were exposed to natural levels of environmental stress over genuine ecological timescales. Furthermore the outcome of the environmental stress (i.e. local extinction events) is known, since the events took place in the past, whereas in recent environments the ultimate impact of the stressor on the community (e.g. adaptation, extinction) is unknown. Those advantages, however, come at the cost of not being able to exactly constrain the main stressor(s).

## 2 Material and methods

### 2.1 Choice of species

In order to characterize the reaction of calcification intensity across a spectrum of ecological preferences, habitats, and multiple extinction events, four species of planktonic Foraminifera were selected in this study. *Globigerinoides ruber* (pink) is a symbiont-bearing species and a strict shallow dweller with a main depth habitat at present of about 20 m in the Eastern Mediterranean (Pujol and Vergnaud Grazzini, 1995). The same depth habitat has been inferred for this species by Rohling et al. (2004) during the deposition of Sapropel S5. Aurahs et al. (2011) have shown that at present, *Globigerinoides ruber* (pink) represents a single genetic type of the *Globigerinoides ruber* s. str. group. Thus the derived weight data are unlikely to be influenced by changes in species ecology or by the presence of multiple cryptic

species with different calcification behaviour. This species appears in the Eastern Mediterranean shortly before the onset of Sapropel S5 deposition and remains in the basin at low relative abundances throughout the sapropel interval (Cane et al., 2002).

In order to observe a reaction in a shallow dwelling symbiont-bearing species to ecological stress, *Orbulina universa* was chosen, because this species exhibits two local extinctions across the studied interval of which at least one is observed basin-wide (Cane et al., 2002). This species has at present a shallow dwelling depth of 20–100 m in the Mediterranean (Pujol and Vergnaud Grazzini, 1995), and Rohling et al. (2004) interpret isotopic signatures in this species as indicative of growth in the summer mixed layer during Sapropel S5. Unlike *G. ruber* (pink), *Orbulina universa* shows a higher degree of cryptic speciation with several known genotypes. Thus, although until now only one genotype of *O. universa* has been reported in the Mediterranean (de Vargas et al., 1999), it cannot be excluded that the derived calcification intensities during the Sapropel S5 were affected by genetic diversity.

In order to extend the observations to deep dwelling species and replicate a response of a species to local extinctions, the species *Globorotalia inflata* and *Globorotalia scitula* have also been studied. Both species show a prominent local extinction at the onset of the Sapropel S5 deposition (Cane et al., 2002). *Globorotalia inflata* is an asymbiotic species dwelling slightly deeper than *O. universa*. Though its main habitat lies above 100 m, it can be found alive at depths up to 700 m (Pujol and Vergnaud Grazzini, 1995; van Raden et al., 2011). It has been interpreted to dwell primarily within the winter mixed layer during the deposition of Sapropel S5 (Rohling et al., 2004). The entire Northern Hemisphere population of this species appears to represent a single genetic type (Morard et al., 2011). In contrast, the asymbiotic *G. scitula* is considered as a deep dwelling species. It is rare in the Mediterranean Sea at present (Pujol and Vergnaud Grazzini (1995) recorded peak abundances of only 9 specimens/1000 $m^3$ of filtered seawater) but analyses in the Atlantic Ocean show that *G. scitula* is most abundant between 200 and 500 m water depth in the Azores region (Schiebel et al., 2002). Rohling et al. (2004) reconstructed a dwelling depth corresponding to the intermediate waters in the Mediterranean, below the summer thermocline, for the time interval during which Sapropel S5 was deposited. The genetic diversity in *G. scitula* has not been studied, yet.

By including the four species in the analysis, our data set can be expected to record the reaction of calcification intensity on both the environmental and biotic forcing across multiple species and timescales of centuries to thousands of years.

### 2.2 Sample processing and environmental data

For this study a portion of gravity core M51-3/SL104 (Hemleben et al., 2003), taken in the Pliny Trench southeast of Crete in the Eastern Mediterranean Sea, was used (Fig. 1). This core contains an exceptionally thick and well-preserved Sapropel S5, which is considered to have been deposited shortly after the Eemian Insolation Maximum (c. 126–121 ka) (Moller et al., 2012). Using a combination of event-based stratigraphy following Cane et al. (2002) with layer counting in the laminated part of the S5 sapropel, Moller (2012) showed that the major part of the studied section at the onset of Sapropel S5 in the studied core recorded an even sedimentation rate of about 4.8 cm $kyr^{-1}$. An abrupt change in the sedimentation rate occurred at 437.2 cm (Fig. 2). This age model is adopted in this study, but for our purposes, the absolute dating is left out, and we report ages relative to the onset of the sapropel.

Across the studied interval, the core was sampled in three-millimetre intervals, which yields a sample resolution of about 60–70 yr in the majority of that section and approximately 11 yr in the topmost 6 cm. Samples were washed over a 63 µm screen and dry-sieved, and only the fractions $\geq$ 150 µm were used for this study. For this study, 70 samples from a section of the core have been selected to cover the onset of Sapropel S5, as well as local extinctions of the studied species within the early part of the sapropel interval (Fig. 2). Specimens for weight analysis have been picked from representative aliquots of the sample. In the majority of the cases, the entire sample has been used (Table S1).

A total of 2025 specimens of *O. universa* were picked from samples 1–70 (455.5–434.8 cm). This interval covers two local extinctions of the species, one in sample 44, after which the community was rapidly re-established, and one in sample 70, after which the species was absent from the sediment record for at least 240 yr. Of *G. scitula* 1290 specimens were picked from samples 1–29 (455.5–447.1 cm), covering an interval until the prominent local extinction from sample 29 to 30, after which the species is absent in the core for at least 360 yr. *Globorotalia inflata* shows a prominent local extinction after sample 31, after which the species remained virtually absent for the remainder of the sapropel. From that interval (455.5–446.5 cm) 4129 specimens of *G. inflata* were picked for analyses. A total of 243 specimens of *G. ruber* (pink) were picked from a narrow size fraction of 180–212 µm from samples 23–59 (448.9–438.1 cm), after the species re-invaded the Eastern Mediterranean.

The transition from sample 29 to 30 (446.95 cm) marks the onset of the sapropel, thus *G. scitula* became extinct shortly before the onset of the sapropel and *G. inflata* immediately afterwards, whereas *O. universa* survived for about 4000 yr after onset of the sapropel (with the exception of sample 44) and *G. ruber* (pink) is hardly present before the sapropel onset at all. Relative abundances of the species were determined from assemblage counts, using 12.5–100 per cent of

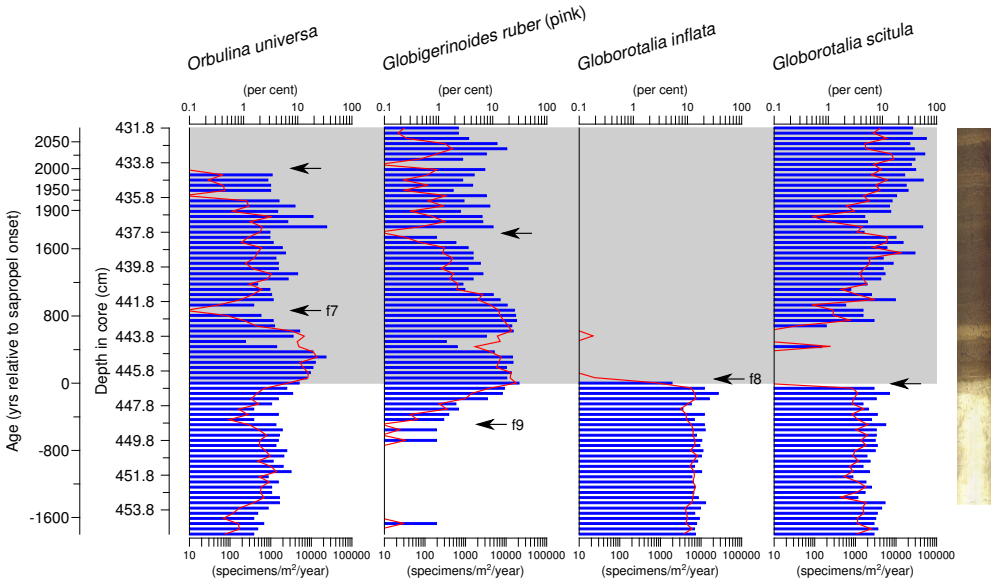



**Fig. 2.** Abundances of the investigated species of planktonic Foraminifera across the onset of Sapropel S5 in core M51-3/SL104, expressed as accumulation rates (blue bars) and relative abundances (red lines). Bioevents that have been used in this study are highlighted with arrows. Labelled arrows refer to bioevents (local extinction of *O. universa* and *G. inflata*, and occurrence of *G. ruber* (pink) in "detectable quantities"), that have been used by Cane et al. (2002, Table 2) to construct the Eastern Mediterranean biostratigraphy for Sapropel S5. The grey shaded area represents the extent of the sapropel. A core photograph is provided for comparison. Ages are given in years relative to sapropel onset. Note that *O. universa* shows no local extinction at 435.7 cm, it only occurs in such small abundances that it was not detected in the split used for abundance reconstructions.

the sample volume (divided with a microsplitter, Table S1); these counts were used to determine absolute abundances, assuming constant sample volume. The relative abundances are considered a proxy for the reproductive success of a species relative to the other species in the planktonic Foraminifera community, and thus provide information about the competitiveness of the species. The absolute abundances could be recalculated to accumulation rates (specimens $m^{-2}\,yr^{-1}$) on the basis of the age model, generating a proxy for species productivity, and thus represent a measure of absolute reproductive success of a species.

For the reconstruction of the hydrological regime, oxygen and carbon stable isotope ratios (VPDB standard) were measured in specimens of *Globigerinoides ruber* (white) taken from the narrow size fraction of 250–315 µm.

### 2.3 Area density

The mean area density (MAD) was determined by weighing the shells using a Mettler Toledo UMX 2 microbalance, and a Sartorius SE 2 for *G. ruber* (pink) (accuracy 0.1 µg for both scales), following procedures for determining the size-normalized weight measurements suggested by Beer et al. (2010a) and Marshall et al. (2013). Shells for weighing were selected by first narrowing the possible size distribution of specimens by means of sieving. For *O. universa* the fraction 425–500 µm, for *G. scitula* and *G.inflata* the 150–200 µm size-fraction, and for *G. ruber* (pink) the 180–212 µm size-fraction was used. All those fractions were chosen as to fall into the peak abundance size of the respective species as best as was possible. The thus selected specimens were cleaned by sonication and dried in a compartment dryer. After drying, specimens were transferred into microslides and left to equilibrate with air moisture for at least 24 h. The specimens were subsequently picked with a needle, discarding all damaged individuals and specimens which showed remains of sediment filling. This process yielded 239 specimens of *O. universa*, 743 specimens of *G. scitula*, 462 specimens of *G. inflata*, and 166 specimens of *G. ruber* (pink) that were suitable for weighing. For the weighing process several shells were placed together in a tin weighing boat and weighed together repeatedly (10–20 times). Following Beer et al. (2010a) we aimed to weigh at least six specimens of *Orbulina universa*, and ten specimens of *Globorotalia scitula*, *Globorotalia inflata*, and *Globigerinoides ruber* (pink), respectively, per sample. After weighing, specimens were mounted on glass slides, using double-sided adhesive tape, photographed with a Leica Z16 stereomicroscope, and their cross-sectional area was measured using either the Image-Pro® Plus v. 6.0 software (Media Cybernetics, Inc., 2006) or FIJI v. 1.47 (Schindelin et al., 2012). For each of the weighed samples, the mean area density (MAD) was determined as the mean measured weight, divided by the number of specimens weighed, normalized for the mean size of the weighed

specimens (Eq. 1).

$$\mathrm{MAD} = \frac{W/n}{S}, \quad (1)$$

where $W$ is the total weight of all specimens $n$ weighed together and $S$ is the mean cross-sectional area of those specimens.

Normalizing the weights for the cross-sectional area potentially introduces a certain error, because shell weight is primarily dependent on shell volume. However, assuming that the form of specimens of the same species remains similar, the resulting error can be considered very small, since shell volume and its cross-sectional area (assuming the same viewpoint was used in all images) are directly proportional. The normalization to area makes comparisons of MAD values among different species impossible. For that purpose we therefore normalized the data to their modern reference samples before comparison (compare Sect. 2.5).

Since the applied weighing procedure provided only one mean weight value per sample, instead of several individual values, confidence intervals for weight measurements could not be calculated by common approaches. To overcome that problem, confidence intervals were estimated by random resampling manually implemented in R v. 2.13.0 (R Development Core Team, 2011). For each investigated species one sample that yielded a relatively large number of weighable specimens was chosen. From that sample, six (*Orbulina universa*) or ten specimens (*Globorotalia scitula*, *Globorotalia inflata*, and *Globigerinoides ruber* (pink)), were randomly picked, weighed together, and then the MAD for that random sample was calculated as described in Eq. (1). The specimens were then put back and the whole procedure was repeated 29 times, yielding 30 partial values for the MAD of 30 random subsamples representative for the variability of the population. Since those partial values were completely random they can be considered to represent individual weights of 30 hypothetical specimens. In the next step, $N$ values from that pool of 30 partial values were randomly chosen (with replacement) and their mean was calculated – this procedure was repeated 2000 times per $N$, for all observed $n$ of the respective species. For each of the random replication sets the 0.025 and 0.975 quantiles were calculated according to the $\hat{Q}_8(p)$ definition recommended by Hyndman and Fan (1996). In that way for each observed sample size $n$ a corresponding 95 % confidence interval for the MAD was approximated for each species. Since the confidence intervals were estimated in this way, the variability of the data could not easily be considered when investigating the relationship between the MAD and other parameters. This problem was dealt with by another randomization test, in which MAD values of the individual samples were randomly chosen from within the range of the 95 % confidence interval. After 5000 reruns the mean of the test statistics was calculated and compared to the test statistics of the original data. Those mean values are marked with a bar hereafter, as opposed to the unmarked statistics of the original measurement values.

*Orbulina universa* is known to consist of multiple cryptic species, so the observed signal could in principle be influenced by a non-constant mixing ratio of different genotypes in the samples. To test for that possibility, specimens of *O. universa* from four selected depths were weighed individually in tin weighing boats (weighing was repeated six times per specimen, and then the mean value was calculated), and similar to the calculation of the MAD the obtained weight was then normalized for the size (cross-sectional area) of that specimen. This provided data sets with several values per depth level. Should any observed signal be the result of a changing genotype composition in the assemblage, we would assume to see the same bi- or multimodal area density distribution in all levels, but with changing amplitudes of the different modes. A unimodal distribution, in which the position of the mode changes with depth level, on the other hand, would indicate a concerted reaction of the entire population irrespective of the number of cryptic species involved. Consequently, Hartigan's Dip Test (Hartigan and Hartigan, 1985) was performed, to test for unimodality in the data.

### 2.4 Data analysis

All statistical tests were conducted in R v. 2.13.0 (R Development Core Team, 2011). Stable isotopic data (i.e. $\delta^{18}$O) of shells of *G. ruber* can be considered to be representative for the environmental change, especially the amount of freshwater inflow at the time of deposition of Sapropel S5. For that reason, a general correlation between stable isotopes and MAD should be tested using independent linear regressions. Several assumptions to use a model I linear regression were violated, so a Kendall–Theil robust line fitting (KTRLF) (Kendall, 1938; Theil, 1950; Sen, 1968) was implemented in R v. 2.13.0 (R Development Core Team, 2011) using equations given in Helsel and Hirsch (2002), and the equation of Conover (1980) to calculate the intercept. Besides being robust against all disturbances as long as values are measured on a meaningful scale, this method also offers the benefit that the confidence intervals of the MAD do not need to be prescribed.

### 2.5 Comparison with reference values from present-day samples

To obtain values for comparison of our results with a representative reference value, we analysed core-top samples (1–0 cm) from multicorer cores, using the same procedures as outlined above. For *Globigerinoides ruber* (pink), *Orbulina universa*, and *Globorotalia inflata* we used a sample from Cruise POS334, Leg 79 from the Western Mediterranean Sea north of Africa (Schulz et al., 2006). This sample has been chosen because it derives from a region where the abundance of these three species is the highest in the

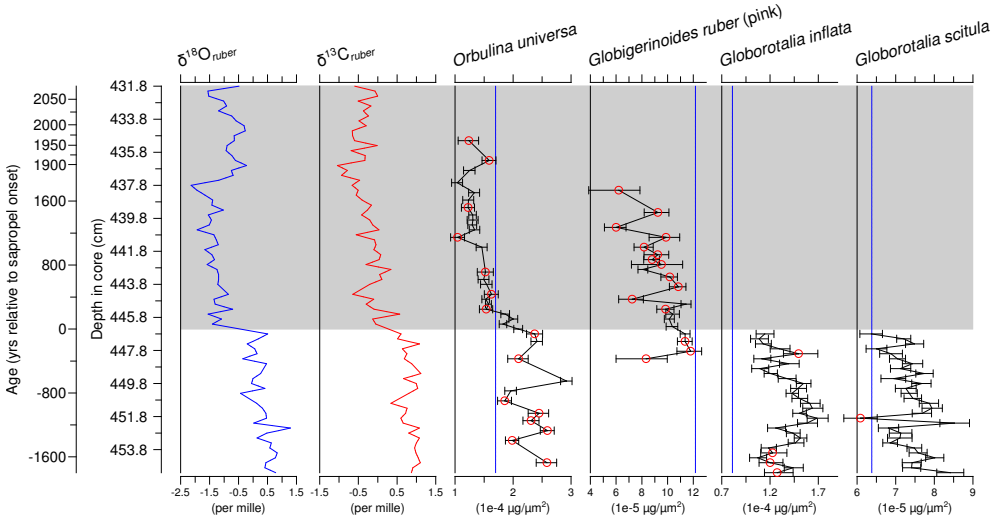



**Fig. 3.** Records of mean area density (MAD) for the four selected species of planktonic Foraminifera across the onset of Sapropel S5 in core M51-3/SL104, with 95 % confidence intervals. Red circles highlight samples where the MAD value is based on fewer specimens ($< 6$ for *Orbulina universa* and $< 10$ for all other species). The blue vertical line shows the MAD value of a modern reference for each species. The grey shaded area represents the extent of the sapropel (Fig. 2). Ages are given in years relative to sapropel onset.

Mediterranean Sea at present (Hayes et al., 2005). *Globorotalia scitula* is not abundant enough anywhere in the Mediterranean today. Modern references for that species were therefore taken from Cruise M34-3, Station 3810-2 (Bleil et al., 1997) from the Southern Atlantic Ocean, halfway between Africa and South America, where the distribution of the species in modern core tops indicates a proximity to its ecological optimum (Kučera et al., 2005). Considering typical sedimentation rates in the vicinity of the two modern samples (3.6 cm kyr$^{-1}$ in the Atlantic (Seiter et al., 2005) and 7.2 cm kyr$^{-1}$ in the Western Mediterranean (Hayward et al., 2009)) and the date when the samples were collected (2006 for POS334 and 1996 for M34-3/3810-2), only a small proportion of the Foraminifera in these samples is likely to have been deposited during the industrial period with $pCO_2$ values more than 20 per cent above the pre-industrial baseline. Therefore, we consider the analysed Foraminifera to be largely representative of pre-industrial $pCO_2$ levels.

Using those recent values as basis for normalization (Eq. 2), MAD values of the different species could be made comparable with each other. Subsequently, Yates $\chi^2$ Test of Association (Yates, 1934) could be used to test the independence of the MAD from environmental factors, and the strength of a potential association could be determined by calculating the $\phi$ Coefficient of Association (Cramér, 1946). For this step only, post-extinction MAD values of *Globorotalia scitula* from two samples from within the sapropel (443.05 and 442 cm) were used to confirm the general trends.

$$\mathrm{MAD_{norm}} = \frac{\mathrm{MAD} - \mathrm{MAD_{recent}}}{s(\mathrm{MAD})}, \quad (2)$$

where $\mathrm{MAD_{norm}}$ is the normalized MAD per sample, on the basis of the recent comparison value $\mathrm{MAD_{recent}}$ and standard deviation $s(\mathrm{MAD})$ of all measured MAD values of the species.

## 3 Results

The relative and absolute abundances of the studied species, together with the position of the regionally established local extinctions and re-populations are shown in Fig. 2. The stable isotope records of *G. ruber* (white), shown in Fig. 3, confirmed a close association of the onset of the sapropel with freshwater discharge and the local extinctions of *G. inflata* and *G. scitula*.

The MAD data for all species are depicted in Fig. 3. *Globigerinoides ruber* (pink) yielded relatively few specimens that were suitable for weighing, so that only 0–20 specimens/sample fulfilled the criteria for weighing (Table S1), with a median sample size of just $\tilde{n} = 4$. The data were normally distributed with $p = 0.275$ according to a Shapiro–Wilk test (Shapiro and Wilk, 1965), with a mean MAD of $9.45 \times 10^{-5}$ µg µm$^{-2}$. Shells of *G. ruber* (pink) were always lighter than in the modern reference, where an MAD of $12.16 \times 10^{-5}$ µg µm$^{-2}$ was determined (Fig. 3). In *O. universa* 2–22 specimens/sample ($\tilde{n} = 6$) fell in the respective size range for weighing (Table S1), yielding MAD data that were not normally distributed ($p = 0.034$). The mean MAD of that species is $1.74 \times 10^{-4}$ µg µm$^{-2}$, which is close to the value derived from the modern references ($1.70 \times 10^{-4}$ µg µm$^{-2}$).

To test for the potential influence of non-constant mixing of different cryptic species of *O. universa* on the mean values

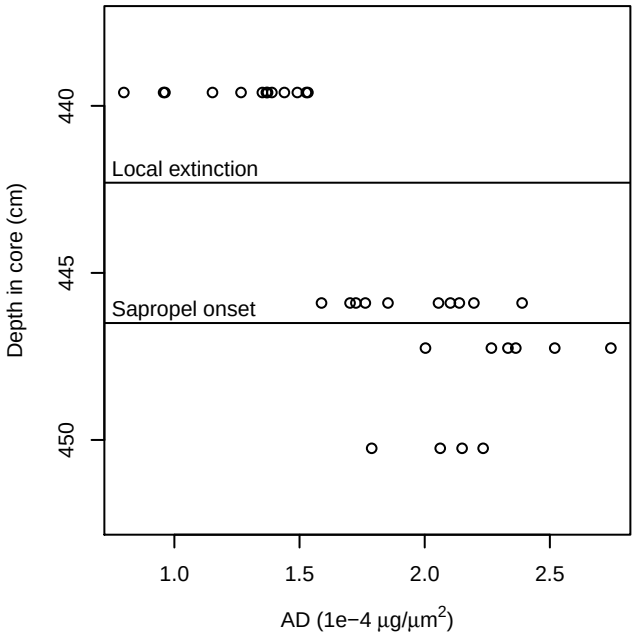



**Fig. 4.** Distribution of area density (AD) values of individual *Orbulina universa* specimens in individual samples across Sapropel S5 in core M51-3/SL104. These samples represent conditions before and after the onset of Sapropel S5 as well as conditions after the first local extinction of the species (Fig. 2). Hartigan's Dip Test (Hartigan and Hartigan, 1985) indicates no significant deviation from unimodality in any of the samples, suggesting that the detected trend of decreasing mean area density over time within the sapropel is not the result of a changing community composition, but a general trend in the overall calcification of the shells of the species.

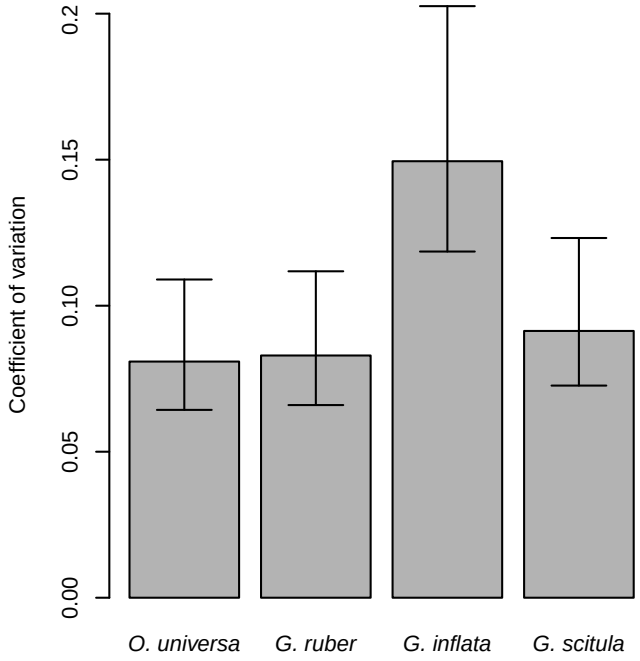



**Fig. 5.** Variance of the mean area density values for the four studied species, expressed as coefficient of variation. The variance is based on replicated measurements of random subsamples from samples 35, 32, 6, and 21 in core M51-3/SL104. The 95 % confidence intervals were estimated after Vangel (1996, Eq. 16).

in the samples, weight measurements of individual specimens for four selected depth levels were performed: samples $17 + 18$ and $27 + 28$ before sapropel onset, sample 32 after sapropel onset, and sample 53 after the first local extinction in sample 44. The data (Fig. 4) for all samples showed a general trend towards lighter shells in the upper samples, with a generally similar variability of the weight. No significant deviation from unimodality could be detected in any of the depth levels ($p_{\min} = 0.240$).

*Globorotalia scitula* yielded between 8 and 50 specimens/sample ($\tilde{n} = 25$) for weighing purposes (Table S1). The MAD was normally distributed ($p = 0.950$), but with a mean MAD of $7.34 \times 10^{-5}\,\mu g\,\mu m^{-2}$ *G. scitula* was the least intensely calcified of the studied species. The MAD of this species in M51-3/SL104 was nearly always higher than in the recent material from the Southern Atlantic ($6.38 \times 10^{-5}\,\mu g\,\mu m^{-2}$).

Between 4 and 31 specimens/sample ($\tilde{n} = 15$) of *G. inflata* were suitable for weighing (Table S1), providing normally distributed MAD data ($p = 0.109$) with a mean of $1.36 \times 10^{-4}\,\mu g\,\mu m^{-2}$. As in *G. scitula*, shells of *G. inflata* were always heavier in the studied samples than in modern Mediterranean sediments ($0.81 \times 10^{-4}\,\mu g\,\mu m^{-2}$). It is worth mentioning, that the variance of the MAD of *G. inflata*, as obtained from the coefficients of variation, was significantly larger than in any of the other species investigated (Fig. 5).

In order to investigate to what degree the obtained MAD mean values are representative of the samples, we took advantage of the data obtained in the procedure to estimate the confidence interval. These data allowed us to estimate the minimum number of specimens to be weighed together to obtain a representative MAD for each species. These data revealed that in all species a change in the slope of the regression between the size of the confidence interval and the number of specimens $n$ considered can be observed. In samples larger than that threshold size, the confidence interval decreased slowly with further increasing sample size in comparison to samples smaller than that threshold size. To determine such threshold values for each species objectively, two regression lines were fitted to the data, one to the steep slope for small sample sizes, one to the shallow slope for larger sample sizes. The border between those two subsets per species was chosen such that the product of the $R^2$ values of both regression lines was minimal (Fig. 6). The resulting threshold sample sizes were $n = 5$ for *O. universa*, $n = 4$ for *G. ruber* (pink), $n = 22$ for *G. scitula*, and $n = 11$ for *G. inflata*. Thus, with the exception of *G. scitula*, the target samples sizes used in this study to determine MAD should yield a good approximation of the MAD value. In *G. scitula*,

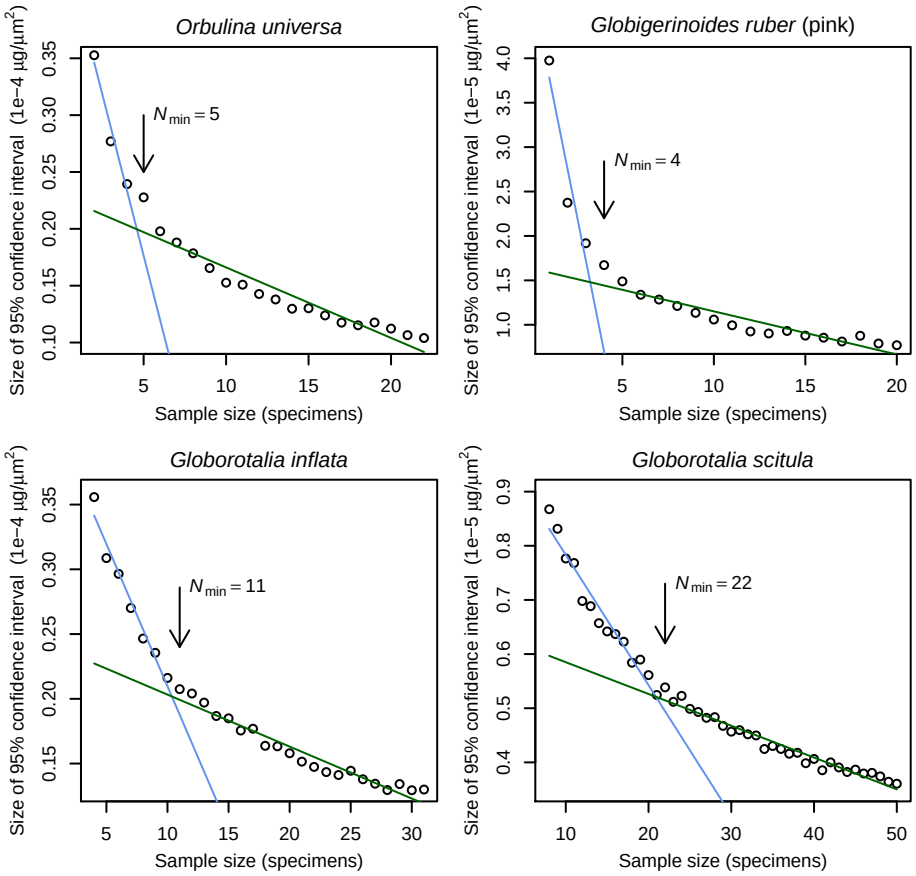



**Fig. 6.** Two-step regression for the estimation of a suitable minimal sample size for mean area density determination in each of the four studied species during the onset of Sapropel S5 in core M51-3/SL104. The two regression lines where chosen such that the product of their respective $R^2$ values is minimal.

a larger sample size would have been desirable to reduce the uncertainty of the MAD values.

## 4 Discussion

### 4.1 Environmental change at the onset of Sapropel S5

The stable isotope curves of *G. ruber* showed a large change with the onset of Sapropel S5. The values and magnitude of change are compatible with hypotheses attributing this phenomenon to the inflow of freshwater from Africa with much lower isotopic values in comparison with seawater (Gasse, 2000; Hoelzmann et al., 2000), due to an enhanced monsoon activity over Africa (Rossignol-Strick, 1983; Rohling et al., 2002; Moller et al., 2012). In response to the freshwater influx, a layer with strongly reduced salinity is assumed to have formed at the top of the water column in the Eastern Mediterranean, causing a stagnation of the vertical circulation and the deposition of Sapropel S5 (Rossignol-Strick et al., 1982; Myers et al., 1998; Rohling et al., 2000, 2009). The collapse of the vertical circulation of the water column occurred very rapidly, within $40 \pm 20$ yr (Marino et al., 2007) after the onset of the elevated freshwater influx. The $\delta^{18}$O stable isotopic signal is found almost coevally in shallow-dwelling and deep-dwelling species of planktonic Foraminifera, including the here-studied *G. scitula* and *G. inflata* (Rohling et al., 2004), indicating that the resulting perturbation of the upper water column affected a major portion of the habitat of planktonic Foraminifera.

While anoxic conditions developed in the deeper water column (e.g. Rinna et al., 2002) the surface waters were subject to different environmental changes. The data indicate that the sea surface temperature rose by about 3 °C during the first 1000 yr after the onset of the sapropel, and remained high for the remainder of sapropel deposition (Marino et al., 2007). Concomitant with the freshwater influx must have been a reduction of surface water salinities towards normal marine values (compare, for instance, Wüst, 1961; Rohling et al., 2009). Because a palaeo-salinity reconstruction on the basis of $\delta^{18}$O is complicated in the Eastern Mediterranean Sea (Rohling et al., 2004), an alternative approach used the isotopic composition of alkenones (van der Meer et al., 2007). This approach indicates a rapid drop in surface

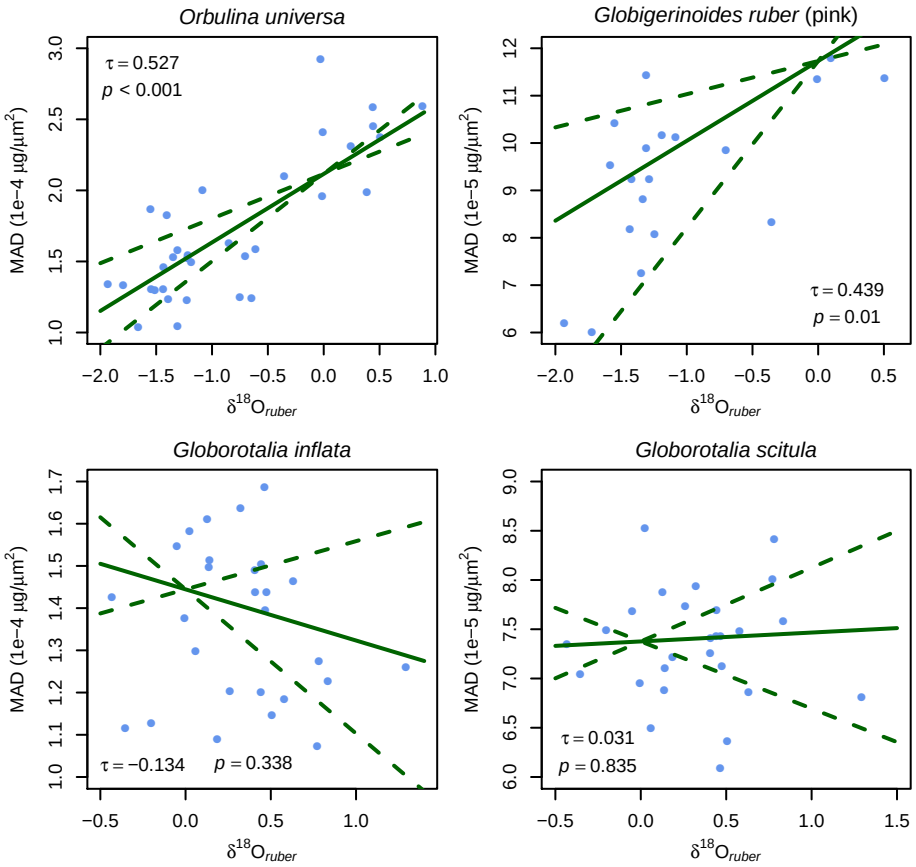



**Fig. 7.** Kendall–Theil robust line fitting (solid lines) of the mean area density (MAD) in the investigated four species with $\delta^{18}$O data of *Globigerinoides ruber* during the onset of Sapropel S5 in core M51-3/SL104. The MAD data are significantly positively correlated with isotopic data for the two shallow-dwelling species. Dashed lines represent the 95 % confidence interval of the regression lines.

water salinity from c. 39 to c. 35 psu with the onset of the sapropel, which then prevailed for about 2000 yr, i.e. for the whole time interval investigated here. Such change in the surface salinity is unlikely to have affected the physiology of the Foraminifera by itself, but it would have led to a large change in carbonate chemistry of the surface water, resulting in decreased calcite saturation (empirical results, e.g. by Trask, 1937; Chierici and Fransson, 2009.)

### 4.2 Abiotic factors vs. physiological stress

In order to test whether abiotic factors have influenced the calcification intensity of the studied Foraminifera, the MAD values were compared to the $\delta^{18}$O record of *Globigerinoides ruber*, which here serves as a proxy of surface water composition. A KTRLF indicates the presence of a significant relationship between $\delta^{18}$O and MAD in *G. ruber* (pink) and *O. universa* (Fig. 7). Additionally, in *O. universa* a significant drop in the MAD from $2.30 \times 10^{-4}$ µg µm$^{-2}$ to $1.44 \times 10^{-4}$ µg µm$^{-2}$ ($\tilde{W} = 257$, $\bar{p} < 0.001$ according to a Mann–Whitney *U* Test) can be observed, which coincides with the fast decrease of stable isotope values in shells of *G. ruber* at the onset of the sapropel. In contrast to those findings, there is no correlation between MAD and stable isotopes detectable in *G. scitula* and *G. inflata*. These species calcify in a deeper layer in the ocean and both exhibit a local extinction at the onset of the sapropel, so the lack of correlation between their calcification intensity and $\delta^{18}$O only refers to the unperturbed conditions prior to the freshwater-induced stratification of the water column.

Although the latter two species cannot provide information on their reaction to environmental change at the onset of the sapropel deposition, their MAD values may provide clues to the general relationship between calcification intensity and surface water chemistry. Because the global sea level was lower than at present during the transitional and deglacial times prior to sapropel deposition, the connection of the Mediterranean Sea with the Atlantic Ocean was more restricted, and the resulting increase in residence time of seawater made the Mediterranean saltier (Rohling, 1999). A comparison of the MAD values of the two deeper-dwelling species indicates that at that time, they built consistently stronger calcified shells than the reference Holocene populations (Fig. 3). A similar pattern is observed for the

**Table 1.** Results of Spearman's Rank-Order Correlation of the mean area density of the species with its abundance. The analysis was performed for the relative abundance (in per cent) and the calculated accumulation rate (in specimens $m^{-2}\,yr^{-1}$).

| Species | Relative abundance $\bar{\rho}$ | Relative abundance $\bar{p}$ value | Accumulation rate $\bar{\rho}$ | Accumulation rate $\bar{p}$ value |
|---|---|---|---|---|
| *O. universa* | 0.149 | 0.397 | 0.085 | 0.631 |
| *G. ruber* (pink) | 0.264 | 0.265 | 0.115 | 0.622 |
| *G. scitula* | 0.024 | 0.804 | −0.075 | 0.692 |
| *G. inflata* | 0.263 | 0.190 | 0.082 | 0.663 |

populations of the two surface-dwelling species in sediments prior to the onset of the sapropel, where *O. universa* also calcifies more than the modern reference, but the pattern is reversed in the sapropel, where both surface-dwelling species seem to have calcified lighter than the reference (Fig. 3). As a result, there is a strong and highly significant relationship between MAD values of all species normalized against their modern references (Eq. 2), and $\delta^{18}$O values of *G. ruber* ($\chi^2 = 70.1393$, $df = 1$, $p < 0.001$, $\phi = -0.825$; Fig. 8). Because of the re-appearance of one of the deep-dwelling species, *Globorotalia scitula*, within the investigated portion of the sapropel (Fig. 2), the implied abiotic forcing of calcification in the studied Foraminifera can be further tested. To this end, two additional samples of this species from within the sapropel have been analysed for MAD. In both cases, the calcification was lighter than the modern reference (Fig. 8), supporting the abiotic forcing hypothesis.

In order to test the alternative hypothesis of calcification intensity being linked to varying levels of physiological stress, the MAD values were compared to changes in the abundances of the species (relative abundance and accumulation rate) as a measure of the suitability of the environment for the species. Close to the environmental optimum of a species its production rates should be highest and according to de Villiers (2004) we should expect to find highest calcification rates under such favourable environmental conditions. However, in no species a significant correlation between the relative abundance or the accumulation rate and the MAD could be detected (Figs. 9 and 10, and Table 1). To assess the existence of such relationship, when all species are considered, in analogy with the concept in Fig. 8, the normalized (Eq. 2) MAD data of all species were plotted against their standardized accumulation rates. In contrast to the high correlation between stable isotopic data and normalized MAD, no correlation between standardized abundances and normalized MAD values could be detected ($\chi^2 = 0.9563$, $df = 1$, $p = 0.328$, $\phi = -0.110$, Fig. 11).

These results indicate that changes in calcification intensity occurred irrespective of changes in productivity of the studied species. Since the magnitude of the considered values of productivity reached all the way to total demise of

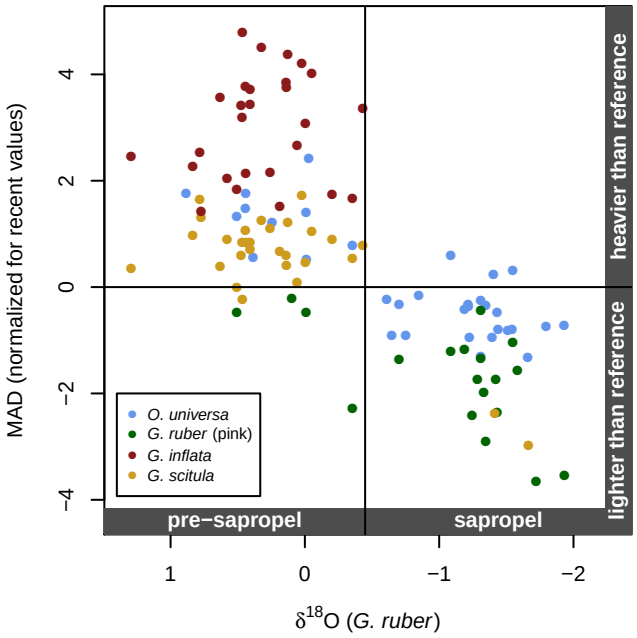



**Fig. 8.** Correlation between normalized (for recent samples) mean area density (MAD) data of the four investigated species and $\delta^{18}$O values of *Globigerinoides ruber* during the onset of Sapropel S5 in core M51-3/SL104. All species reflect a consistent pattern of stronger calcification during times of higher isotopic values.

the species, the results indicate that calcification in planktonic Foraminifera is de-coupled from environmental stress and likely not affected by trade-offs between biomineralization and biomass production. This decoupling is observed at a timescale of centuries, leaving a possibility that the reaction of calcification on physiological stress could be a short-term, threshold process, operating first at near-lethal levels of stress such as would be expected immediately prior to extinction. To this end, we have examined the MAD values in the last samples prior to local extinctions (Fig. 2) in all four species. None of the MAD values of the last sample prior to extinction were the lowest values for the respective species in the studied interval (Fig. 3). This observation indicates either an absence of a notable effect of stress on calcification or the existence of such a relationship only at timescales of a few decades or less, that could not be resolved by our sampling.

### 4.3 Factors influencing calcification intensity in planktonic Foraminifera

The observed trends in calcification intensity of the studied species are very unlikely to be the result of changing carbonate preservation throughout the time interval. Due to the high salinities in the Eastern Mediterranean, the seawater saturation with respect to calcite has likely remained high (Schneider et al., 2007), so that no calcite dissolution should be expected. In fact, from our observation the preservation of Foraminifera within the sapropel was better than

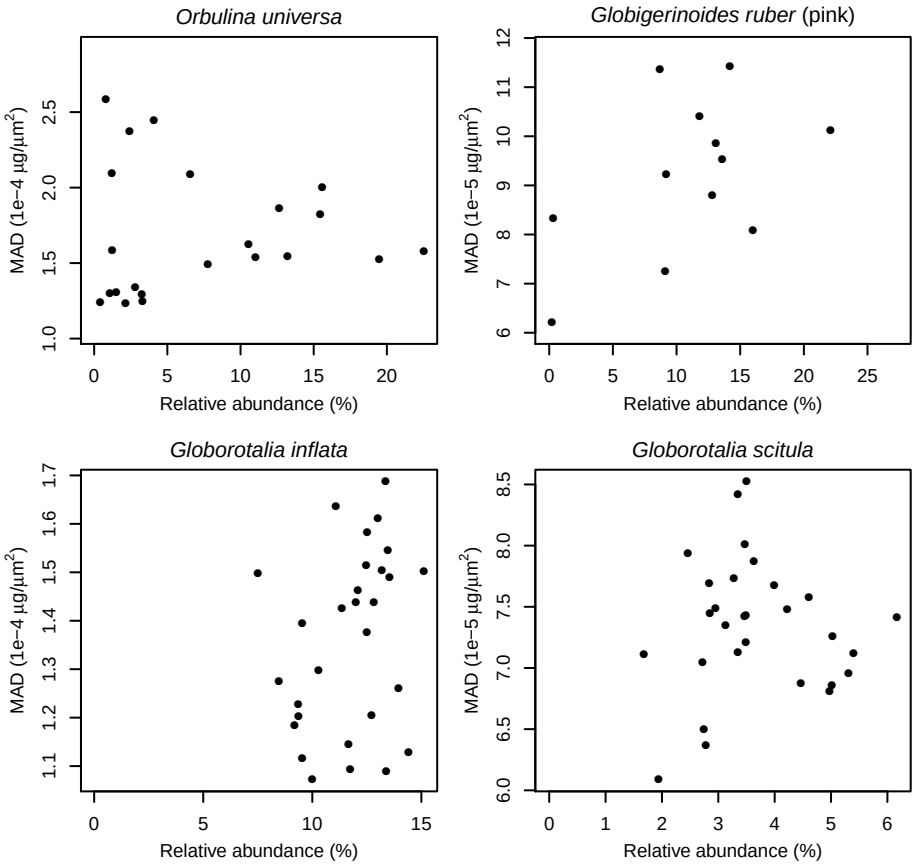



**Fig. 9.** Correlation between relative abundance and mean area density (MAD) of the four investigated species. No significant correlation can be observed, indicating that the calcification rate and competitiveness of the species were independent of each other during the onset of Sapropel S5 in core M51-3/SL104.

before the sapropel, with pristine, glassy specimens of even tiny thin-walled species. This effect is directly comparable to the "sealing" effect in hemipelagic sediments as described by Pearson et al. (2001). The final evidence against carbonate dissolution as a factor affecting the measured MAD is the continuous presence throughout the studied interval of pteropods – which are very sensitive to carbonate dissolution due to their aragonitic mineralogy.

In addition to dissolution, the precipitation of inorganic secondary calcite in pre-sapropel samples could also influence our results. The precipitation of secondary calcite in the pre-sapropel sediment, however, is unlikely because all specimens appear clean and well preserved even before the sapropel, and the MAD values of *G. ruber* (pink) in pre-sapropel sediments are similar to the modern reference. If secondary inorganic precipitation affected the MAD values, it should have done so equally strongly for all species, because such a process is inorganic and could not be species-selective.

Of the studied species, *Orbulina universa* is known to harbour three distinct cryptic genetic types. If these genetic types, which likely represent biological species (de Vargas et al., 1999), calcify differently, the observed trend in the species could reflect a change in their relative proportions. This possibility has been investigated by individually weighing specimens from four selected time horizons (Table S2, Fig. 4). These data indicate a shift of the entire population towards lower calcification intensities, with kernel density curves for each of the four levels not significantly different from unimodality. While this is no proof for a constant community composition, it shows that our calculated MAD values represent a reaction of the whole community, not a switch from one bimodal state to another. The pre- and post-sapropel-onset MAD distributions are so distinct that if they were solely due to differences in the calcification among different cryptic genetic types, then these values alone could be easily used to distinguish among them, which is difficult to reconcile with their cryptic nature.

Excluding the effect of carbonate dissolution and genotype abundance fluctuations on the observed decrease in MAD with the onset of the sapropel, calls for an explanation involving the effect of shifts in the parameters of the ambient water column. *Orbulina universa* shows the strongest reaction to the changing environment, with a clear drop in MAD at the onset of the sapropel, a continuous decrease of calcification

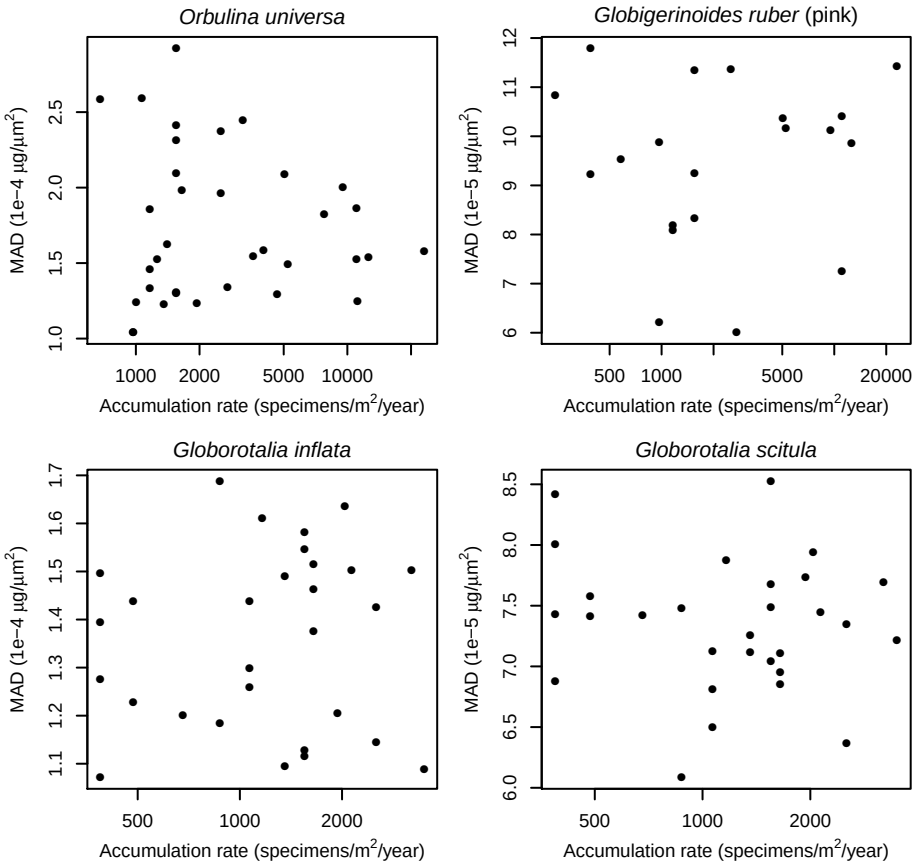



**Fig. 10.** Correlation between accumulation rates and mean area density (MAD) of the four investigated species. No significant correlation can be observed, indicating that the calcification intensity and productivity of the species were not influenced by each other during the onset of Sapropel S5 in core M51-3/SL104. Note the log-scaling of the $x$ axis.

intensity within the sapropel, and a strong relationship with stable isotopic data and thus probably freshwater influx. *Globigerinoides ruber* (pink) shows similar results, though the drop in MAD with sapropel onset could not be clearly tested because the species was not abundant enough at that time. Both species calcify in the upper water column (Pujol and Vergnaud Grazzini, 1995; Rohling et al., 2004) and were therefore strongly influenced by the freshwater inflow, thriving in a water mass with presumably reduced salinity in comparison to normal Eastern Mediterranean conditions (van der Meer et al., 2007). Because multiple parameters changed in parallel during the onset of the sapropel, it is difficult to isolate the primary abiotic factor that may have led to reduced calcification intensity in these species.

Obviously, the freshwater influx have not caused reduced carbonate production, since Moller et al. (2012) reported a short-lasting rise in Ca content in the sediment from 13.9 to 29.9 per cent with the onset of the sapropel, followed by a return to pre-sapropel conditions, decoupled from changes in MAD of the shallow-dwelling species.

Therefore, we entertain the possibility that the observed reduction in calcification intensity could be due to changes in seawater carbonate chemistry. Such a link seems to be the most commonly invoked hypothesis explaining differences in calcification rates among planktonic Foraminifera (Barker and Elderfield, 2002). Because it may be assumed that $\delta^{18}$O of *G. ruber* was correlated with surface salinity during the onset of Sapropel S5 (Gasse, 2000; Hoelzmann et al., 2000), the close link between this variable and the MAD of the studied species is consistent with a dominant forcing by carbonate chemistry of the ambient seawater, in line with the observations by Bijma et al. (1999), Barker and Elderfield (2002), and Marshall et al. (2013). Higher carbonate saturation states at the time before sapropel deposition, as suggested by higher MAD values (Fig. 3), were most likely the result of higher salinity in the glacial water body in the Eastern Mediterranean Sea, which developed because of longer residence times due to the lower sea level restricting water exchange with the open ocean (Rohling, 1999).

The lack of a relationship between MAD in *G. inflata* and *G. scitula*, and $\delta^{18}$O of *G. ruber* in the interval prior to sapropel deposition likely reflects the deeper calcification depth of those species, compared to *O. universa*. We hypothesize that the subsurface layer, where the calcification

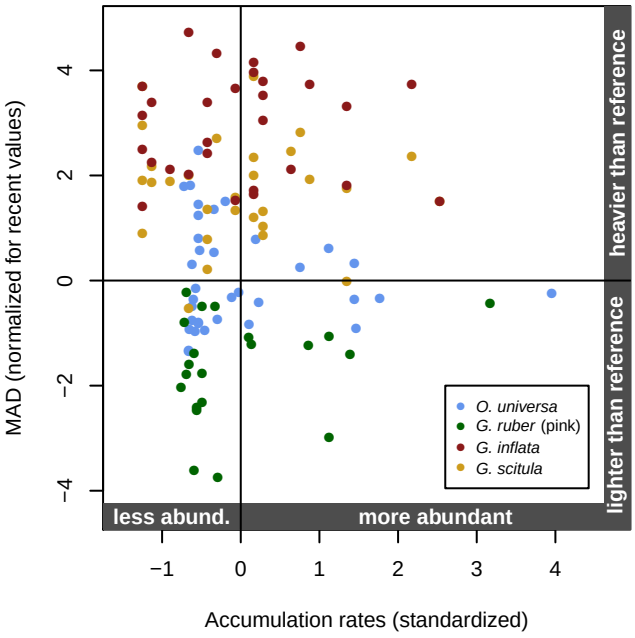



**Fig. 11.** The relationship between normalized (for recent samples) mean area density (MAD) data of the four investigated species and their standardized accumulation rates during the onset of Sapropel S5 in core M51-3/SL104. The lack of correlation indicates that calcification intensity is not related to stress on the studied timescale.

of these species is likely to have been concentrated (Rohling et al., 2004), experienced a weaker reaction to environmental changes prior to sapropel deposition than the surface layer. This possibility is also suggested by results from Rohling et al. (2004, Fig. 5). This study from the same time interval and region shows, that the general trend of reduced stable isotopic values can be found in shell calcite of a variety of planktonic Foraminifera, including *G. scitula*. However, the deeper dwelling species show isotopic values indicating that their habitat was less affected by the freshwater discharge. In accordance with our results (compare Fig. 8) this suggests, that the environmental change leading to the deposition of Sapropel S5 affected the deeper water column as well, but to a lesser degree. A broader calcification depth in *G. inflata*, not limited to the surface layer, is supported by the larger variability in calcification intensity in that species (Fig. 5).

In summary, the analysis using normalized calcification intensity data of all species together strongly supports a dominant abiotic forcing of calcification intensity in planktonic Foraminifera at timescales of decades to centuries. While all species support a relationship between proxies of surface water properties and MAD, no correlation between MAD and abundance of the studied species could be observed. This relation indicates a strong influence of the environmental change itself on calcification in all species, but no influence of the stress associated with that change, that would be reflected in the changing productivity of a species as inferred from its accumulation rates. This is further supported by the complete lack of a reaction of calcification intensity on terminal environmental stress leading to local extinction in any of the species investigated in this study. These results support the use of calcification intensity in planktonic Foraminifera as an environmental proxy. Even if we could not isolate, which environmental factors acted on the calcification process in which combination, it is likely that the process of calcification is not complicated by insurmountably complex biological relationships, and the observations in this study are consistent with the hypothesis that calcification in planktonic Foraminifera is driven by carbonate chemistry of the ambient water.

## 5 Conclusions

Our study has shown that the calcification intensity in four species of planktonic Foraminifera was likely related to environmental changes during the onset of Sapropel S5 in the Eastern Mediterranean. Specifically, we observe significant relationships between MAD and a proxy of surface water properties, and no relationship between calcification and productivity of the studied species.

Concerning our hypotheses we can thus state, that we were only able to observe long-term changes associated with abiotic factors of the environmental change (hypothesis a1). Neither long-term reactions of the calcification intensity and optimal growth conditions (hypothesis a2) nor short-term reactions of the calcification intensity during times of terminal environmental stress (hypothesis b) could be observed in our data set. This observation supports the use of the calcification intensity of planktonic Foraminifera as a palaeoproxy for environmental reconstructions. Even though the exact combination of environmental factors acting on calcification of planktonic Foraminifera during the natural experiment of the onset of Sapropel S5 could not be disentangled, the observed patterns are consistent with calcification intensity being driven by carbonate chemistry of the ambient seawater.

**Supplementary material related to this article is available online at http://www.biogeosciences.net/10/6639/2013/bg-10-6639-2013-supplement.zip.**

*Acknowledgements.* The authors want to thank the responsible editor Tina Treude and two anonymous referees for their constructive comments on the discussions version of this article. The authors acknowledge the financial aid of the Land Baden–Württemberg (granted in the form of the Landes-Graduierten-Förderungs-Gesetz – LGFG), which provided the financial basis for the principal author. Tobias Moller received funding through the German Research Foundation DFG-grant SCHU1605/3-1. We are thankful

to Hartmut Schulz and Barbara Donner for providing the material of the modern references, and Agnes Weiner for help with the final editing of the manuscript. The others members of the Minigraduiertenprojekt in Tübingen are acknowledged for their invaluable help and support in many situations, which cannot be listed in detail.

Edited by: T. Treude

## References

Aldridge, D., Beer, C. J., and Purdie, D. A.: Calcfication in the planktonic Foraminifera *Globigerina bulloides* linked to phosphate concentrations in surface waters of the North Atlantic Ocean, Biogeosciences, 9, 1725–1739, doi:10.5194/bg-9-1725-2012, 2012.

Aurahs, R., Treis, Y., Darling, K., and Kučera, M.: A revised taxonomic and phylogenetic concept for the planktonic foraminifer species *Globigerinoides ruber* based on molecular and morphometric evidence, Mar. Micropaleontol., 79, 1–14, doi:10.1016/j.marmicro.2010.12.001, 2011.

Barker, S. and Elderfield, H.: Foraminiferal calcification response to glacial–interglacial changes in atmospheric $CO_2$, Science, 297, 833–836, doi:10.1126/science.1072815, 2002.

Beer, C. J., Schiebel, R., and Wilson, P. A.: Technical note: On methodologies for determining the size-normalised weight of planktic Foraminifera, Biogeosciences, 7, 2193–2198, doi:10.5194/bg-7-2193-2010, 2010a.

Beer, C. J., Schiebel, R., and Wilson, P. A.: Testing planktic foraminiferal shell weight as a surface water $[CO_3^{2-}]$ proxy using plankton net samples, Geology, 38, 103–106, doi:10.1130/G30150.1, 2010b.

Bijma, J., Spero, H. J., and Lea, D. W.: Reassessing foraminiferal stable isotope geochemistry: Impact of the oceanic carbonate system (experimental results), in: Use of Proxies in Paleoceanography: Examples from the South Atlantic, edited by: Fischer, G. and Wefer, G., 489–512, Springer-Verlag, Berlin, Heidelberg, 1999.

Bleil, U., Brück, L., Frederichs, T., Haese, R., Hensen, C., Hilgenfeldt, C., Hoek, R., Hübscher, C., von Lom-Keil, H., Janke, A., Kreutz, R., Keenan, J., Little, M. L., Martens, H., Rosiak, U., Schmidt, W., Schneider, R., Segl, M., Spieß, V., Uenzelmann-Neben, G., Urbanek, H., and Zühlsdorff, L.: Geo Bremen South Atlantic 1996, METEOR Berichte, Leitstelle METEOR, Hamburg, 1997.

Broecker, W. and Clark, E.: An evaluation of Lohmann's Foraminifera weight dissolution index, Paleoceanography, 16, 531–534, doi:10.1029/2000PA000600, 2001.

Cane, T., Rohling, E. J., Kemp, A. E. S., Cooke, S., and Pearce, R. B.: High-resolution stratigraphic framework for Mediterranean Sapropel S5: Defining temporal relationships between records of Eemian climate variability, Palaeogeogr. Palaeocl. Palaeoecol., 183, 87–101, doi:10.1016/S0031-0182(01)00461-8, 2002.

Chierici, M. and Fransson, A.: Calcium carbonate saturation in the surface water of the Arctic Ocean: Undersaturation in freshwater influenced shelves, Biogeosciences, 6, 2421–2432, doi:10.5194/bg-6-2421-2009, 2009.

Conover, W. J.: Practical Nonparametric Statistics, Wiley Series in Probability and Mathematical Statistics, John Wiley & Sons, Ltd., New York, 1980.

Cramér, H.: Mathematical Methods of Statistics, vol. 9 of *Princeton Mathematical Series*, Princeton University Press, Princeton, 1946.

de Moel, H., Ganssen, G. M., Peeters, F. J. C., Jung, S. J. A., Kroon, D., Brummer, G. J. A., and Zeebe, R. E.: Planktic foraminiferal shell thinning in the Arabian Sea due to anthropogenic ocean acidification?, Biogeosciences, 6, 1917–1925, doi:10.5194/bg-6-1917-2009, 2009.

de Vargas, C., Norris, R., Zaninetti, L., Gibb, S. W., and Pawlowski, J.: Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces, P. Natl. Acad. Sci. USA, 96, 2864–2868, doi:10.1073/pnas.96.6.2864, 1999.

de Villiers, S.: Optimum growth conditions as opposed to calcite saturation as a control on the calcification rate and shell-weight of marine Foraminifera, Mar. Biol., 144, 45–49, doi:10.1007/s00227-003-1183-8, 2004.

Gasse, F.: Hydrological changes in the African tropics since the last glacial maximum, Quaternary Sci. Rev., 19, 189–211, doi:10.1016/S0277-3791(99)00061-X, 2000.

Hartigan, J. A. and Hartigan, P. M.: The Dip Test of unimodality, Ann. Stat., 13, 70–84, doi:10.1214/aos/1176346577, 1985.

Hayes, A., Kučera, M., Kallel, N., Sbaffi, L., and Rohling, E. J.: Glacial Mediterranean sea surface temperatures based on planktonic foraminiferal assemblages, Quaternary Sci. Rev., 24, 999–1016, doi:10.1016/j.quascirev.2004.02.018, 2005.

Hayward, B. W., Sabaa, A. T., Kawagata, S., and Grenfell, H. R.: The Early Pliocene re-colonisation of the deep Mediterranean Sea by benthic Foraminifera and their pulsed Late Pliocene–Middle Pleistocene decline. Mar. Micropal., 71, 97–112, doi:10.1016/j.marmicro.2009.01.008, 2009.

Helsel, D. R. and Hirsch, R. M.: Statistical methods in water resources, in: Hydrologic Analysis and Interpretation, vol. 4 of *Techniques of Water-Resources Investigations Reports*, chap. A3, 1–510, US Geological Survey, 2002.

Hemleben, C., Becker, T., Bellas, S., Benningsen, G., Casford, J., Cagatay, N., Emeis, K.-C., Engelen, B., Ertan, T., Fontanier, C., Friedrich, O., Frydas, D., Giunta, S., Hoffelner, H., Jorissen, F., Kahl, G., Kaszemeik, K., Lykousis, V., Meier, S., Nickel, G., Overman, J., Pross, J., Reichel, T., Robert, C., Rohling, E., Ruschmeier, W., Sakinc, M., Schiebel, R., Schmiedl, G., Schubert, K., Schulz, H., Tischnak, J., and Truscheit, T.: Ostatlantik–Mittelmeer–Schwarzes Meer Part 3: Cruise No. 51, Leg 3, METEOR Berichte, Leitstelle METEOR, Hamburg, 2003.

Hoelzmann, P., Kruse, H.-J., and Rottinger, F.: Precipitation estimates for the eastern Saharan palaeomonsoon based on a water balance model of the West Nubian Palaeolake Basin, Global Planet. Change, 26, 105–120, doi:10.1016/S0921-8181(00)00038-2, 2000.

Hyndman, R. J. and Fan, Y.: Sample quantiles in statistical packages, Am. Stat., 50, 361–365, doi:10.1080/00031305.1996.10473566, 1996.

Kendall, M. G.: A new measurement of rank correlation, Biometrika, 30, 81–93, doi:10.1093/biomet/30.1-2.81, 1938.

Kučera, M., Weinelt, M., Kiefer, T., Pflaumann, U., Hayes, A., Weinelt, M., Chen, M.-T., Mix, A. C., Barrows, T. T., Cor-

tijo, E., Duprat, J., Juggins, S., and Waelbroeck, C.: Reconstruction of sea-surface temperatures from assemblages of planktonic Foraminifera: Multi-technique approach based on geographically constrained calibration data sets and its application to glacial Atlantic and Pacific Oceans, Quaternary Sci. Rev., 24, 951–998, doi:10.1016/j.quascirev.2004.07.014, 2005.

Lohmann, G. P.: A model for variation in the chemistry of planktonic Foraminifera due to secondary calcification selective dissolution, Paleoceanography, 10, 445–457, doi:10.1029/95PA00059, 1995.

Lombard, F., da Rocha, R. E., Bijma, J., and Gattuso, J.-P.: Effect of carbonate ion concentration and irradiance on calcfication in planktonic Foraminifera, Biogeosciences, 7, 247–255, doi:10.5194/bg-7-247-2010, 2010.

Manno, C., Morata, N., and Bellerby, R.: Effect of ocean acidification and temperature increase on the planktonic foraminifer *Neogloboquadrina pachyderma* (sinistral), Polar Biol., 35, 1311–1319, doi:10.1007/s00300-012-1174-7, 2012.

Marino, G., Rohling, E. J., Rijpstra, W. I. C., Sangiorgi, F., Schouten, S., and Damsté, J. S. S.: Aegean Sea as driver of hydrographic and ecological changes in the Eastern Mediterranean, Geology, 35, 675–678, doi:10.1130/G23831A.1, 2007.

Marshall, B. J., Thunell, R. C., Henehan, M. H., Astor, Y., and Wejnert, K. R.: Planktonic foraminiferal area density as a proxy for carbonate ion concentration: A calibration study using the Cariaco Basin Ocean Time Series, Paleoceanography, 28, 1–14, doi:10.1002/palo.20034, 2013.

Media Cybernetics, Inc.: Image-Pro® Plus Version 6.0 for Windows™ Start-Up Guide, Silver-Spring, 2006.

Moller, T.: Formation and palaeoclimatic interpretation of a continuously laminated Sapropel $S_5$: A window to the climate variability during the Eemian interglacial in the Eastern Mediterranean, Ph.D. thesis, Eberhard–Karls-Universität Tübingen, Tübingen, http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-63524, 2012.

Moller, T., Schulz, H., Hamann, Y., Dellwig, O., and Kučera, M.: Sedimentology and geochemistry of an exceptionally preserved last interglacial Sapropel S5 in the Levantine Basin (Mediterranean Sea), Mar. Geol., 291–294, 34–48, doi:10.1016/j.margeo.2011.10.011, 2012.

Morard, R., Quillévéré, F., Douady, C. J., de Vargas, C., de Garidel-Thoron, T., and Escarguel, G.: Worldwide genotyping in the planktonic foraminifer *Globoconella inflata*: Implications for life history and paleoceanography, PLoS ONE, 6, e26665, doi:10.1371/journal.pone.0026665, 2011.

Moy, A. D., Howard, W. R., Bray, S. G., and Trull, T. W.: Reduced calcification in modern Southern Ocean planktonic Foraminifera, Nat. Geosci., 2, 276–280, doi:10.1038/NGEO460, 2009.

Myers, P. G., Haines, K., and Rohling, E. J.: Modeling the paleocirculation of the Mediterranean: The last glacial maximum and the Holocene with emphasis on the formation of Sapropel $S_1$, Paleoceanography, 13, 586–606, doi:10.1029/98PA02736, 1998.

Pearson, P. N., Ditchfield, P. W., Singano, J., Harcourt-Brown, K. G., Nicholas, C. J., Olsson, R. K., Shackleton, N. J., and Hall, M. A.: Warm tropical sea surface temperatures in the Late Cretaceous and Eocene epochs, Nature, 413, 481–487, doi:10.1038/35097000, 2001.

Pujol, C. and Vergnaud Grazzini, C.: Distribution patterns of live planktic foraminifers as related to regional hydrography and productive systems of the Mediterranean Sea, Mar. Micropaleontol., 25, 187–217, doi:10.1016/0377-8398(95)00002-I, 1995.

R Development Core Team: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, http://www.R-project.org/, 2011.

Rinna, J., Warning, B., Meyers, P. A., Brumsack, H.-J., and Rullkötter, J.: Combined organic and inorganic geochemical reconstruction of paleodepositional conditions of a Pliocene sapropel from the Eastern Mediterranean Sea, Geochim. Cosmochim. Ac., 66, 1969–1986, doi:10.1016/S0016-7037(02)00826-8, 2002.

Rohling, E., Abu-Zied, R., Casford, J., Hayes, A., and Hoogakker, B.: The marine environment: Present and past, in: The Physical Geography of the Mediterranean, edited by: Woodward, J., The Oxford Regional Environments Series, chap. 2, 33–67, Oxford University Press, New York, 2009.

Rohling, E. J.: Environmental control on Mediterranean salinity and $\delta^{18}O$, Paleoceanography, 14, 706–715, doi:10.1029/1999PA900042, 1999.

Rohling, E. J., de Rijk, S., Myers, P. G., and Haines, K.: Palaeoceanography and numerical modelling: The Mediterranean Sea at times of sapropel formation, in: Climates: Past and Present, edited by: Hart, M. B., vol. 181 of *Special Publications of the Geological Society*, 135–149, Geological Society, London, doi:10.1144/GSL.SP.2000.181.01.13, 2000.

Rohling, E. J., Cane, T. R., Cooke, S., Sprovieri, M., Bouloubassi, I., Emeis, K.-C., Schiebel, R., Kroon, D., Jorissen, F. J., Lorre, A., and Kemp, A. E. S.: African monsoon variability during the previous interglacial maximum, Earth Planet. Sc. Lett., 202, 61–75, doi:10.1016/S0012-821X(02)00775-6, 2002.

Rohling, E. J., Sprovieri, M., Cane, T., Casford, J. S. L., Cooke, S., Bouloubassi, I., Emeis, K.-C., Schiebel, R., Rogerson, M., Hayes, A., Jorissen, F. J., and Kroon, D.: Reconstructing past planktic foraminiferal habitats using stable isotope data: A case history for Mediterranean Sapropel S5, Mar. Micropaleontol., 50, 89–123, doi:10.1016/S0377-8398(03)00068-9, 2004.

Rossignol-Strick, M.: African monsoons, an immediate climate response to orbital insolation, Nature, 304, 46–49, doi:10.1038/304046a0, 1983.

Rossignol-Strick, M., Nesteroff, W., Olive, P., and Vergnaud Grazzini, C.: After the deluge: Mediterranean stagnation and sapropel formation, Nature, 295, 105–110, doi:10.1038/295105a0, 1982.

Schiebel, R., Waniek, J., Zeltner, A., and Alves, M.: Impact of the Azores Front on the distribution of planktic foraminifers, shelled gastropods, and coccolithophorids, Deep-Sea Res. Pt. II, 49, 4035–4050, doi:10.1016/S0967-0645(02)00141-8, 2002.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., and Cardona, A.: Fiji: An open-source platform for biological-image analysis, Nat. Methods, 9, 676–682, doi:10.1038/nmeth.2019, 2012.

Schmidt, D. N., Renaud, S., Bollmann, J., Schiebel, R., and Thierstein, H. R.: Size distribution of Holocene planktic foraminifer assemblages: Biogeography, ecology and adaptation, Mar. Micropaleontol., 50, 319–338, doi:10.1016/S0377-8398(03)00098-7, 2004.

Schneider, A., Wallace, D. W. R., and Körtzinger, A.: Alkalinity of the Mediterranean Sea, Geophys. Res. Lett., 34, L15608, doi:10.1029/2006GL028842, 2007.

Schulz, H., Bayer, M., Denker, C., Hemleben, C., Martínez Botí, M., Numberger, L. D., Palamenghi, L., Storz, D., and van Raden, U.: Cruise Report – Cruise No.: 334, F. S. Poseidon Cruise Reports, Leibniz-Institut für Meereswissenschaften an der Universität Kiel, Kiel, 2006.

Seiter, K., Hensen, C., and Zabel, M.: Benthic carbon mineralization on a global scale. Global Biogeochem. Cy., 19, GB1010, doi:10.1029/2004GB002225, 2005.

Sen, P. K.: Estimates of the regression coefficient based on Kendall's Tau, J. Am. Stat. Assoc., 63, 1379–1389, http://www.jstor.org/stable/2285891, 1968.

Shapiro, S. S. and Wilk, M. B.: An analysis of variance test for normality (complete samples), Biometrika, 52, 591–611, doi:10.1093/biomet/52.3-4.591, 1965.

Theil, H.: A rank-invariant method of linear and polynomial regression analysis, III, P. K. Ned. Akad. Wetensc., 53, 1397–1412, 1950.

Trask, P. D.: Relation of salinity to the calcium carbonate content of marine sediments, in: Shorter Contributions to General Geology, edited by: Ickes, H. I. and Mendenhall, W. C., vol. 186 of *United States Geological Survey Professional Papers*, 273–299, United States Government Printing Office, Washington, https://play.google.com/store/books/details?id=P0IRAAAAIAAJ&rdid=book-P0IRAAAAIAAJ&rdot=1, 1937.

van der Meer, M. T. J., Baas, M., Rijpstra, W. I. C., Marino, G., Rohling, E. J., Damsté, J. S. S., and Schouten, S.: Hydrogen isotopic compositions of long-chain alkenones record freshwater flooding of the Eastern Mediterranean at the onset of sapropel deposition, Earth Planet. Sc. Lett., 262, 594–600, doi:10.1016/j.epsl.2007.08.014, 2007.

van Raden, U. J., Groeneveld, J., Raitzsch, M., and Kučera, M.: Mg/Ca in the planktonic Foraminifera *Globorotalia inflata* and *Globigerinoides bulloides* from Western Mediterranean plankton tow and core top samples, Mar. Micropaleontol., 78, 101–112, doi:10.1016/j.marmicro.2010.11.002, 2011.

Vangel, M. G.: Confidence intervals for a normal coefficient of variation, Am. Stat., 50, 21–26, http://www.jstor.org/stable/2685039, 1996.

Williams, D. F., Thunell, R. C., and Kennett, J. P.: Periodic freshwater flooding and stagnation of the Eastern Mediterranean Sea during the late Quaternary, Science, 201, 252–254, doi:10.1126/science.201.4352.252, 1978.

Wüst, G.: On the vertical circulation of the Mediterranean Sea, J. Geophys. Res., 66, 3261–3271, doi:10.1029/JZ066i010p03261, 1961.

Yates, F.: Contingency tables involving small numbers and the $\chi^2$ Test, J. Roy. Stat. Soc. Supp., 1, 217–235, http://www.jstor.org/stable/2983604, 1934.

# Chapter 5

**Manuscript 2**: Weinkauf, M. F. G., Kunze, J. G., and Kučera, M. (in preparation) Seasonal variation in shell calcification of planktonic Foraminifera in the NE Atlantic reveals species-specific response to temperature, productivity, and optimum growth conditions, *Biogeosciences*

## Abstract

Using shells collected from a sediment trap series in the Azores region of the North Atlantic, we investigate the effects of seasonal variation of temperature, productivity, and optimum growth conditions on calcification in three species of surface-dwelling planktonic Foraminifera. The series covers an entire seasonal cycle at three-week resolution and reflects conditions at the edge of the distribution of the studied species, manifesting more suitable growth conditions during different parts of the year. The seasonal variability in carbonate saturation at the studied site is much smaller than in previous studies of calcification in planktonic Foraminifera, allowing us to disentangle the effect of parameters other than carbonate saturation. In order to better constrain the calcification process, we use weight and size data collected from individual shells. We find that the size–weight scaling within each species is robust against changes in environmental parameters, but we observe that the scaling slope differs among species. An analysis of the variation in calcification intensity, expressed as the average value of the area density of individual shells in each sample, reveals species-specific response patterns. In *Globigerinoides ruber* (white) and *Globigerinoides elongatus*, calcification intensity is related with temperature (positive) and productivity (negative), whilst in *Globigerina bulloides* calcification intensity shows no environmental forcing. The size–weight scaling and base calcification intensity as well as the response of calcification intensity to environmental change differed between *G. ruber* (white) and *G. elongatus*, implying that patterns extracted from pooled analyses of these species may reflect their changing proportions in the pooled samples. Using shell flux as a measure of optimum growth conditions, we observe significant positive correlation with calcification intensity only in *G. elongatus*, but a negative relationship in *G. bulloides*. The lack of a consistent response to optimum growth conditions is mirrored by analysis of shell sizes in these species. We thus conclude that calcification intensity in planktonic Foraminifera is affected by factors other than carbonate saturation, but the strength and even the sign of the relationship with temperature, productivity and optimum growth conditions are not consistent among species, potentially complicating interpretations of calcification data from the fossil record.

Manuscript prepared for Biogeosciences
with version 2014/07/29 7.12 Copernicus papers of the LaTeX class copernicus.cls.
Date: 23 March 2015

# Seasonal variation in shell calcification of planktonic Foraminifera in the NE Atlantic reveals species-specific response to temperature, productivity, and optimum growth conditions

**M. F. G. Weinkauf**[1,2], **J. G. Kunze**[1], **and M. Kučera**[2]

[1]Eberhard–Karls University Tübingen, Hölderlinstr. 12, 72074 Tübingen, Germany
[2]MARUM, University Bremen, Leobener Str., 28359 Bremen, Germany

*Correspondence to:* M. F. G. Weinkauf (mweinkauf@marum.de)

**Abstract.** Using shells collected from a sediment trap series in the Azores region of the North Atlantic, we investigate the effects of seasonal variation of temperature, productivity, and optimum growth conditions on calcification in three species of surface-dwelling planktonic Foraminifera. The series covers an entire seasonal cycle at three-week resolution and reflects conditions at the edge of the distribution of the studied species, manifesting more suitable growth conditions during different parts of the year. The seasonal variability in carbonate saturation at the studied site is much smaller than in previous studies of calcification in planktonic Foraminifera, allowing us to disentangle the effect of parameters other than carbonate saturation. In order to better constrain the calcification process, we use weight and size data collected from individual shells. We find that the size–weight scaling within each species is robust against changes in environmental parameters, but we observe that the scaling slope differs among species. An analysis of the variation in calcification intensity, expressed as the average value of the area density of individual shells in each sample, reveals species-specific response patterns. In *Globigerinoides ruber* (white) and *Globigerinoides elongatus*, calcification intensity is related with temperature (positive) and productivity (negative), whilst in *Globigerina bulloides* calcification intensity shows no environmental forcing. The size–weight scaling and base calcification intensity as well as the response of calcification intensity to environmental change differed between *G. ruber* (white) and *G. elongatus*, implying that patterns extracted from pooled analyses of these species may reflect their changing proportions in the pooled samples. Using shell flux as a measure of optimum growth conditions, we observe significant positive correlation with calcification intensity only in *G. elongatus*, but a negative relationship in *G. bulloides*. The lack of a consistent response to optimum growth conditions is mirrored by analysis of shell sizes in these species. We thus conclude that calcification intensity in planktonic Foraminifera is affected by factors other than carbonate saturation, but the strength and even the sign of the relationship with temperature, productivity and optimum growth conditions are not consistent among species, potentially complicating interpretations of calcification data from the fossil record.

## 1 Introduction

Planktonic Foraminifera are important marine calcifiers, contributing 30–80 % to the global pelagic carbonate flux (e.g. Kučera, 2007). Considering the importance of planktonic Foraminifera for the global carbon cycle, the processes controlling how much calcite is secreted during the life of an individual remain poorly constrained. Calcification in planktonic Foraminifera is an energy-consuming process (Robbins, 1988; Spero, 1988), making it likely that it participates in trade-offs of energy allocation within the cell. Thus, in theory, several environmental parameters could act in favour of calcification, including seawater chemistry (e.g. carbonate saturation) and ambient temperature but also the distance from the ecological optimum of a species as a measure of how much energy is free for calcification and how much is needed to facilitate biomass growth under sub-optimal conditions.

Most often, the amount of calcification in planktonic Foraminifera has been correlated with the physical and chemical properties of their environment, but there is broad disagreement about the dominant controlling parameters and

even about the nature of their relationship with calcification. Based on field observations (Lohmann, 1995; Broecker and Clark, 2001a; Barker and Elderfield, 2002; de Moel et al., 2009; Moy et al., 2009; Marshall et al., 2013) and laboratory culturing studies (Bijma et al., 1999; Lombard et al., 2010), carbonate saturation state of the ambient seawater appears to be the most promising parameter to explain variations in the amount of calcification in planktonic Foraminifera. However, an analysis of plankton samples from the Arabian Sea (Beer et al., 2010b) revealed that the shape of the relationship between carbonate saturation and shell weight is species-specific and its sign is not always positive. Subsequently, culturing experiments (Manno et al., 2012) have shown that the effect of carbonate chemistry on shell calcification in Foraminifera is also a function of temperature. Similarly, a study based on Pliocene sediments (Davis et al., 2013) also found no link between calcification in planktonic Foraminifera and atmospheric $pCO_2$ as a proxy for carbonate chemistry, but rather identified temperature as a potential factor explaining the observed variability in foraminiferal shell calcification. Because both parameters are tightly linked, it is challenging to disentangle their relative contributions even in well constrained studies based on recent sediment-trap material (Marshall et al., 2013).

Carbonate chemistry and temperature are not the only variables invoked to explain changes in calcification of planktonic Foraminifera. Thus, based on plankton material from the North Atlantic, Aldridge et al. (2012) identified phosphate concentration in the ambient sea water as the potential dominant factor influencing the calcification of *Globigerina bulloides*. Conversely, de Villiers (2004) proposed that shell calcification in planktonic Foraminifera could be linked to growth under optimal environmental conditions, meaning that shell calcification is highest when the combination of all environmental factors is close to the optimum of the species. A similar relationship was suggested for the calcification of *Globigerinoides ruber* in sediment trap samples from the Arabian Sea (Naik et al., 2013). Also, growth under optimum conditions has been invoked as the best predictor of the overall mean shell size of specimens within species of planktonic Foraminifera (Hecht, 1976; Schmidt et al., 2004). Assuming that optimal environmental conditions are mirrored in the absolute and relative abundances of a species (with higher abundances indicating more optimal environments), the relationship between optimum growth and calcification has been tested by Weinkauf et al. (2013) in fossil samples from a Mediterranean sapropel. This study found no evidence for a relationship between calcification and ecological optimum, but identified changes in seawater properties as the most likely parameter affecting calcification.

A pre-requisite for any investigation of factors controlling calcification in planktonic Foraminifera is a definition of a meaningful measure of the amount of calcite precipitated by one individual. This quantity can be easily determined as the weight of the shell, but it reflects two parameters: calcification intensity and shell size. In order to use shell weight as a proxy for calcification intensity, one could thus either normalize weight by size or determine the weight of shells of equal sizes. Traditionally, calcification intensity in planktonic Foraminifera has been quantified by using a parameter known as size-normalized weight (SNW), which is a compromise between the two possible strategies. Its most simple form is the sieve-based weight (SBW; e.g. Lohmann, 1995; Broecker and Clark, 2001a, b) where multiple individuals in a narrow size fraction are weighed together and then the mean of their weight is determined. A more advanced version is the measurement-based weight (MBW; e.g. Barker and Elderfield, 2002; Aldridge et al., 2012), where the SBW is normalized for the actual measured mean individual shell size of the specimens in the weighed size fraction. In theory, when the actual size of the measured individuals is determined, the measure of calcification intensity does not have to be limited to a narrow size fraction and the calcification of an individual shell can be directly normalized to its size, typically approximated by the cross-sectional area of the shell (area density, AD; e.g. Marshall et al., 2013) and then averaged per sample.

All of these approaches make one critical assumption: that calcification intensity is independent of shell size. In plankton samples, an additional assumption is made: that the measured specimens all represent an equivalent ontogenetic stage. This additional assumption arises from the observation of increased calcification with ontogeny (Bé and Lott, 1964) and renders data from plankton samples potentially difficult to interpret. In sediment trap samples and in the sediment, the majority of the deposited shells represent adult individuals that have undergone the same ontogenetic pathway (Erez and Honjo, 1981). However, sedimentary individuals attributable to the same species vary in size considerably and it has never been established how calcification intensity scales across the analysed range of shell sizes. Until now, all studies have assumed that calcification intensity is invariant to size and considered 'mean' calcification intensity within one size range to be representative for all individuals in the analysed population. However, if calcification intensity varies with size, the interpretation of calcification intensity data based on such 'mean' values will be ambiguous (Fig. 1). This assumption can be easily tested by determining the relationship between shell weight and size among individual shells across a range of sizes for individual samples. If there is no change in calcification intensity with size, the relationship will be linear (as long as size is scaled to volume), the residuals will be small, and the slope of the linear regression will be the same across all samples studied.

This study therefore aims at specifically testing for the stability of the relationship between calcification intensity and size across species and under different environmental conditions, and at contributing to the understanding of factors other than carbonate saturation affecting calcification intensity in planktonic Foraminifera. Sediment samples, which in-

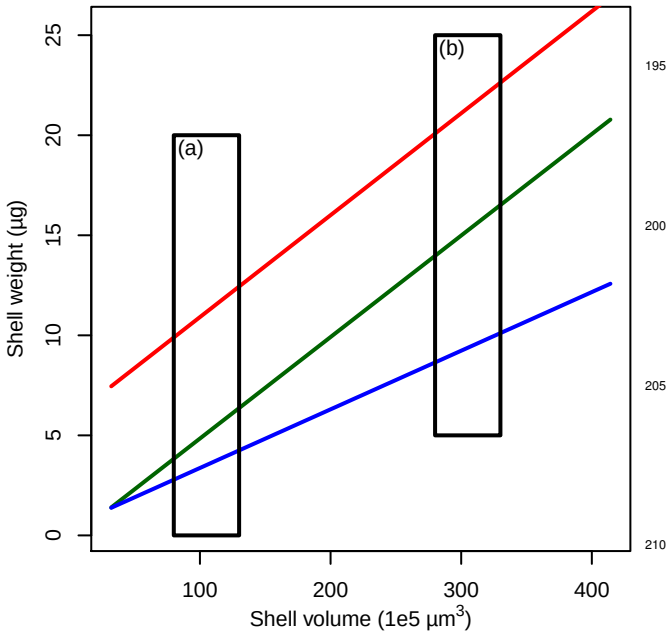



**Figure 1.** Conceptual figure illustrating the difficulties of interpreting the SNW when the size–weight scaling is not constant. Lines depict the size–weight scaling from three hypothetical communities (colour-coded), boxes (a) and (b) are two possible samples from restricted size fractions of those communities. Within the green and the red assemblage, the scaling remains constant at $0.05\,\mu g\,1 \times 10^{-5}\,\mu m^{-3}$. Here, the chosen size fraction for measuring the calcification intensity does not influence the results, since the offset between both lines is constant for all shell sizes. The blue assemblage, however, shows a lower scaling slope of only $0.03\,\mu g\,1 \times 10^{-5}\,\mu m^{-3}$. Here the observed difference in calcification intensity with both the green and red assemblage would be larger in size fraction (b) than they are in size fraction (a).

tegrate shell flux over several years, are not suitable for such a task, because the observed patterns of shell size and weight relationships within the sample could not be attributed to forcing. Similarly, analyses of plankton material will be dominated by the ontogenetic process, bearing little relevance for studies of the fossil record. Therefore, we consider sediment trap samples as optimum choice for such an analysis, providing both high temporal resolution with adequate sample sizes and a limitation of the sample to specimens equivalent to those as found in the sediment. Specifically, we use material collected with a sediment trap in the North Atlantic Madeira Basin close to the Azores Front (Fig. 2a). The Azores Front is situated in the northeastern Atlantic, resulting from the Azores Current that flows towards east-southeast as a branch of the North Atlantic Current (Klein and Siedler, 1989). The Azores Front is separating the cooler regions to the north from the warm North Atlantic Subtropical Gyre (Longhurst, 1995) to the south (Locarnini et al., 2013). Due to the annual variability in the position of the Azores Current, the catchment area of the studied sediment trap shows a large seasonal cycle in surface conditions. The local sea surface temperature (SST) ranges between $c.17$–$18\,^{\circ}C$ early in the year and $c.24$–$25\,^{\circ}C$ during late summer and autumn, with eutrophic late winter–early spring conditions (January–March) and an oligotrophic summer and autumn (Waniek et al., 2005). While no actual data for the carbonate saturation of the seawater in our study exist, we could use average data for temperature (Locarnini et al., 2013), salinity (Zweng et al., 2013), phosphate and silicate content (Garcia et al., 2013), and total $CO_2$ and alkalinity (Goyet et al., 2000) to calculate the average seasonal variation of $\left[CO_3^{2-}\right]$ during the year. The average seasonal carbonate saturation in the catchment area of the sediment trap (compare Waniek et al., 2005, fig. 5) ranges from $214.1\,\mu mol\,kg^{-1}$ in spring to less than $215.3\,\mu mol\,kg^{-1}$ in winter and summer, and only reaches peak values of $220.4\,\mu mol\,kg^{-1}$ in autumn (calculated with CO2Sys, MS Excel v. 2.1, Lewis et al., 1998). The regional carbonate system is nearly exclusively influenced by temperature, with salinity only contributing to slightly more than $10\,\%$ to the total carbonate saturation change.

The average three-weeks sampling resolution assures that each sample represents the deposition of one or very few generations of Foraminifera (Bé, 1977), that were exposed during life to equivalent, near-constant environmental conditions. Because the sampling covers one entire seasonal cycle, we can investigate how the size-weight relationship in multiple species behaves under different temperatures and under different conditions relative to the optimum of those species. Varying on average by no more than $7\,\mu mol\,kg^{-1}$, the carbonate saturation at the studied locality changes only by a small amount, allowing us to study the effects of temperature and ecological optimum independent of carbonate chemistry. Using this natural experiment, we can use measurements of individual shell size and weight to determine the stability of the size–weight relationship and assess the effect of several environmental factors on the calcification intensity of planktonic Foraminifera whilst accounting for that effect.

## 2 Material and methods

### 2.1 Sample collection and preparation

This study is based on material collected by the JGOFS trap 53 from station L1/276, located around 33° N and 22° W in the Madeira Basin, in direct vicinity to the Azores Front, with a local ocean depth of approximately $5500\,m$ at the mooring (Fig. 2). The trap has been deployed at about $2000\,m$ depth, sampling between February 2002 and April 2003 with variable sampling duration (ranging between 6 and 61 days), adapted to the expected seasonal particle flux (Suppl. 1). A total of 18 sample cups were used for our study. Information on trap design, sample treatment, and physical oceanography during deployment are reported in Waniek et al. (2005);

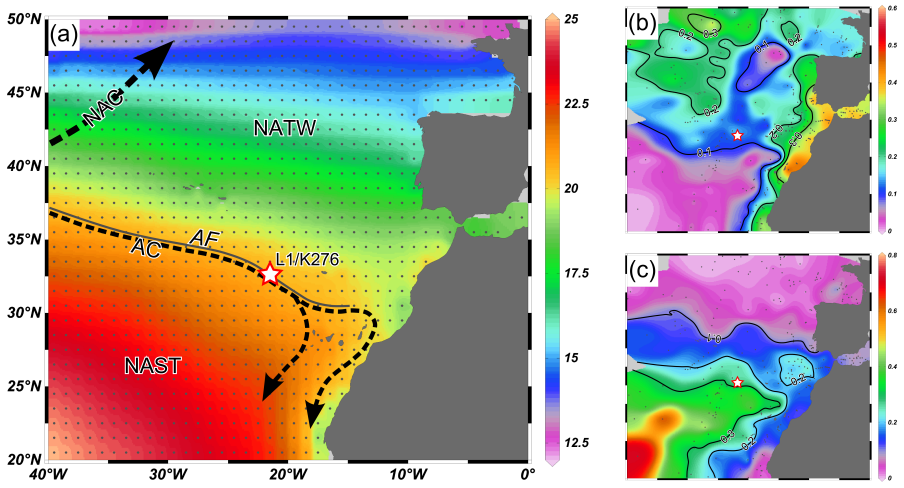



**Figure 2.** Regional setting and abundance pattern (MARGO database, Prell et al., 1999a, b) of investigated species around sediment trap L1/K276 (red–white star), plotted with Ocean Data View v. 4.6.2 (Schlitzer, 2014). Original data points used for interpolation are indicated with grey dots, light grey indicates areas with no data. **(a)** Annual mean sea surface temperature (SST, Locarnini et al., 2013) and main ocean currents of the region. The trap is situated at the Azores Front (AF), in direct vicinity to the Azores Current (AC). The Azores Front separates the North Atlantic Transitional Water (NATW) with mean SST below 20 °C from the North Atlantic Subtropical Gyre (NAST) with a mean SST above 20 °C. NAC = North Atlantic Current. **(b)** The relative abundance (fraction) of *Globigerina bulloides* shows local abundances between 10 and 20 % in the area, which is at the lower end of the northward increasing regional mean abundance of that species north of the Azores Front. **(c)** The relative abundance (fraction) of *Globigerinoides ruber* (white) sensu MARGO includes specimens of *Globigerinoides elongatus*, for which no separate census counts exist in the database. Mean abundances of the morphospecies are approximately 30 %, and it is thus approaching the borders of its distribution area, which shows higher abundances further to the south.

sample processing for analysis of planktonic Foraminifera assemblage composition is described in Storz et al. (2009). Only the fraction $> 150\,\mu m$ (separated by dry-sieving) was used for this study.

## 2.2 Choice of species

The species for this study were chosen to represent a broad environmental spectrum while at the same time occurring in sufficient abundances to provide suitable sample sizes. *Globigerinoides ruber* (white) is a symbiont bearing species that is bound to the upper water column due to the photosynthetic activity of its symbionts. It was shown to be highly abundant throughout the year, with peak abundances of up to 40 % of the total community of planktonic Foraminifera between July and January (Storz et al., 2009). The activity of the symbionts in *G. ruber* (white) may buffer environmental effects otherwise influencing the ability of the species to calcify its shells. Therefore, *Globigerina bulloides* has been selected as the second species for this study. This species does not possess symbionts, but shares a similar depth habitat with *G. ruber* (white) in the studied region (maximum abundances occur above 100 m water depth (Schiebel et al., 2002)). Distribution of *G. ruber* (white) and *G. bulloides* in the sediment (Fig. 2b–c) indicates that the position of the sediment trap for both species is close to their ecological limits. Their abundance in the trap series as already reported by Storz et al. (2009) is consistent with this observation: *Globigerinoides ruber* (white) is more abundant in the warmer subtropical regions whereas *G. bulloides* is a temperate species. The morphological groups *G. ruber* s.str. (inflated chambers in the last whorl) and *G. ruber* s.lat. (compressed chambers in the last whorl) have been recognized within *G. ruber* (white) (e.g. Wang, 2000). In a combined morphological and genetic investigation, Aurahs et al. (2011) have shown that the morphotype *G. ruber* s.lat. represents a different species. Following the criteria in Aurahs et al. (2011), we use the name *G. ruber* (white) for specimens of the morphotype *G. ruber* s.str., and include in our analysis a third species *Globigerinoides elongatus* that refers to specimens of the *G. ruber* s.lat. morphotype. The ecology of *G. elongatus* has not been studied in detail, but it appears that the species has a slightly broader ecological range than *G. ruber* (white) and it appears to calcify deeper in the water column (Steinke et al., 2005; Numberger et al., 2009).

## 2.3 Data acquisition

Specimens of the species *G. ruber* (white), *G. elongatus*, and *G. bulloides* were picked from the $> 150\,\mu m$ fraction in all samples and transferred into cardboard slides for further processing. The flux of the three species was calculated by divid-

ing their observed abundances in the $> 150\,\mu m$ fraction by the opening area of the trap ($0.5\,m^2$, Kremling et al., 1996) and by the sampling duration (in days) of the individual samples (Suppl. 1). We did not use the flux published in Storz et al. (2009), because there fluxes for the fraction $> 125\,\mu m$ are given and no distinction between *G. ruber* (white) and *G. elongatus* is made. As a result, the flux presented here is much smaller (up to $85\,\%$) than that shown in Storz et al. (2009), but this is consistent with the fact that the majority of specimens in all species (on average $50\,\%$ in *Globigerinoides* and $60\,\%$ in *G. bulloides*) is between $125\,\mu m$ and $150\,\mu m$ in size (Storz, 2006). When qualitatively comparing our fluxes with those from Storz et al. (2009) a Spearman rank-order correlation shows a highly significant correlation ($p < .001$) with correlation coefficients of $\rho > 0.8$, further indicating the correctness of our data.

The size of all specimens of the three species has been determined such that the specimens were photographed in umbilical view under constant magnification using a Leica Z16 stereomicroscope equipped with a $5\,MPx$ industrial camera and the photographs were analysed with the Image-Pro Plus software (Media Cybernetics, Inc., 2006). As size parameters, the length of the longest shell-axis (Feret diameter) and the cross-sectional area of the shell were extracted from the images of all shells.

To determine the calcification intensity, all specimens of the three species within a certain size range were individually weighed. For *G. ruber* (white) and *G. elongatus*, all specimens from the $200–250\,\mu m$ size range were measured, to obtain values best comparable with previous investigations (compare Beer et al., 2010a). For *G. bulloides* the $200–300\,\mu m$ size range was used in order to obtain more individuals and to investigate the linearity of the size–weight relationship across a broader size range. Individual shells were transferred into tin weighing boats and repeatedly (4–5 times) weighed with a Mettler Toledo UMX 2 microbalance. The mean value of the repeated measurements was used to represent the weight of each shell. This procedure also allowed calculating the standard error of the weight measurements. The measurements were repeated to alleviate the effects of drift and external disturbance during the weighing process.

Because the weight of the Foraminifera was close to the lower end of the measurement range, the accuracy of the measurements can be expected to be a function of weight, with presumably higher relative accuracy in heavier objects. To quantify this effect, we used specimens of *G. ruber* (white) and *G. elongatus* from the richest sample (cup number three; second half of March 2002, 42 specimens, Suppl. 1) to determine the relationship between individual standard errors (from repeated measurements of the same specimen) and the mean weight of that specimen. We found that lower weights indeed show higher relative standard errors (Fig. 3). While the relative standard error of the measurement is well below $4\,\%$ for the majority of the shells, it can rise up to $8\,\%$ for specimens lighter than $4\,\mu g$. Since nearly $75\,\%$ of all weighed individuals are heavier than $4\,\mu g$ (Suppl. 1), the resulting mean relative error is below $5\,\%$. On the basis of the weight ($W_i$) and the cross-sectional area of the shell ($A_i$), the individual area density ($AD_i$) per specimen could be calculated as $AD_i = A_i/W_i$.

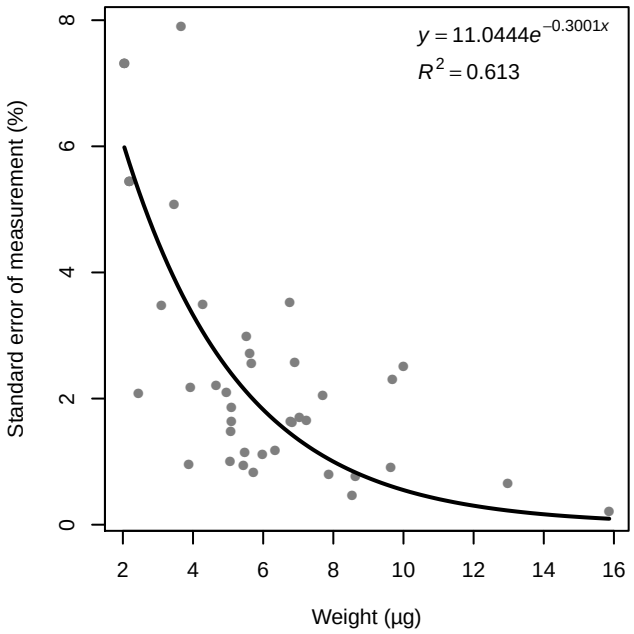



**Figure 3.** Relative standard error of weight measurements as function of mean weight of the specimen, using a Mettler Toledo UMX 2 microbalance ($d = 0.1\,\mu g$). The measurement error can reach relatively high values for weights below $4\,\mu g$, and generally decreases with increasing weight (following a negative expontential function).

Environmental data were retrieved from online repositories. Monthly sea surface temperature (SST) and surface salinity (SSS) data were taken from the KNMI Climate Explorer website (http://climexp.knmi.nl/), using the ICOADS 2°dataset (NOAA/OAR/ESRL PSD) for SST and the UKMO EN3 analysis (Ingleby and Huddleston, 2007) for SSS. Weekly surface Chlorophyll a concentrations of the surface water were retrieved from the U.S. Joint Ocean Flux Study (Yoder and Kennelly, 2005). All data were averaged for the approximated catchment area ($31.65°–35.70°$ N, $19.51°–26.96°$ W Waniek et al., 2005, fig. 5) of the sediment trap and the sampling interval of the respective sample.

All raw data used in this study are provided in Supplement 1.

### 2.4 Data analysis

All statistical analyses of the data were conducted using the software R v. 3.1.0 (R Development Core Team, 2014). Confidence intervals for sample means of shell size and calcification intensity were calculated by bootstrapping using the R-package 'boot' v. 1.3-10 (Davison and Hinkley, 1997).

As suggested by Dixon (2002) we used basic bootstrapping when the data showed a significant skewness, and accelerated bootstrapping when they did not. Skewness was considered significant when the skewness calculated according to Tabor (2010, equation I, table 1) was larger than its approximated standard deviation. The normality of data distribution was tested with a Shapiro–Wilk test (Shapiro and Wilk, 1965), while the homoscedasticity of data was tested with a Fligner–Killeen test (Fligner and Killeen, 1976).

To test the linearity of the size–weight regression within samples, the shell cross-sectional area was first scaled to volume by taking the area to the power of $3/2$. The thus transformed size data were used as the independent variable in a Kendall–Theil robust line fitting (Kendall, 1938; Theil, 1950; Sen, 1968), implemented in R on the basis of equations from Helsel and Hirsch (2002), against the dependent variable shell weight. The slope (including its $95\,\%$ confidence interval) of the resulting regression line and the strength of the relationship (coefficient of determination $R^2$) were calculated. Since not all species were abundant enough in all samples to yield significant results, we only used the slopes of regressions that were significant at $\alpha = .05$ in the ensuing analyses. To determine whether or not the relationship between size and weight is linear within the investigated size range, each linear regression model was tested against an exponential model using the $F$-distribution (McDonald, 2009).

Differences in size–weight scaling among species were analysed using a Kruskal–Wallis test (Kruskal and Wallis, 1952), with ensuing pairwise Mann–Whitney $U$ tests (Mann and Whitney, 1947) (with $p$-values corrected for the false discovery rate according to Benjamini and Hochberg (1995)). To test for the influence of environmental parameters on the stability of the size–weight relationship, we used a robust multiple linear regression between the regression slopes against multiple candidate controlling variables on the basis of the MM-estimate (Yohai et al., 1991), as implemented in the R-package 'robust' v. 0.4-15. To test for the influence of environmental parameters on the calcification intensity of the shells of each species, we applied a variety of generalized linear models (GLM) (Nelder and Wedderburn, 1972). The models were ranked using the corrected Akaike information criterion ($\mathrm{AIC}_c$, Akaike, 1974) calculated with the R-package 'bbmle' v. 1.0.16, and the best model was used for further interpretation.

## 3 Results

### 3.1 Fluxes of the analysed species

The shell fluxes in the fraction larger than $150\,\mu\mathrm{m}$ are shown in Figure 4. *Globigerina bulloides* showed lower mean flux ($5.01\,\mathrm{specimens\,m^{-2}\,day^{-1}}$) than *G. ruber* (white) combined with *G. elongatus* ($11.02\,\mathrm{specimens\,m^{-2}\,day^{-1}}$). The flux of *G. ruber* (white) and *G. elongatus* were on average rather similar.

Throughout the sampling period, all three species showed highest flux between March and May 2002, and generally lower flux during all other months, including March and April 2003; though *G. elongatus* displays a second peak in flux between August and September 2002 (Fig. 4). From March to June 2002, *G. bulloides* generally showed the highest flux of all species, followed by *G. ruber* (white) and *G. elongatus*. For the rest of the investigated time interval the flux values were generally reversed, with *G. elongatus* mostly showing the highest flux before *G. ruber* (white) and *G. bulloides* (Fig. 4).

### 3.2 Shell size

Shell sizes (Feret diameter, Fig. 5) in *Globigerina bulloides* range between $162.7\,\mu\mathrm{m}$ and $446.4\,\mu\mathrm{m}$ (mean: $257.2\,\mu\mathrm{m}$). Although on average the size distribution of *G. ruber* (white) and *G. elongatus* combined is similar, ranging from $146.2\,\mu\mathrm{m}$ to $449.3\,\mu\mathrm{m}$ (mean: $245.0\,\mu\mathrm{m}$), *G. ruber* (white) (mean: $221.3\,\mu\mathrm{m}$, Fig. 5b) is generally smaller than *G. elongatus* (mean: $267.0\,\mu\mathrm{m}$, Fig. 5c). Shells of both *Globigerinoides* species are smallest during late winter and early spring and largest in March–April and around July. In contrast, *G. bulloides* shows smallest shell sizes in early to midsummer (June–July) and relatively large shells during the rest of the year (Fig. 5a). In all species the shell size distribution is log-normal and unimodal in the vast majority of samples (Suppl. 2, Table S1), indicating the presence of only one statistical population.

### 3.3 Shell calcification

The relationship between size (scaled to volume) and weight of individual shells in representative samples is shown in Fig. 6. In all samples and for all species, we observe strong linear relationships between the two variables, indicating a constant scaling between size and weight within the studied size range. To test this conclusion explicitly, we determined the exponential regression through the same points and checked for a significant increase in $R^2$. The $R^2$-value could be significantly increased in $c.14\,\%$ of the cases, but was decreased in nearly $35\,\%$ of the cases by fitting an exponential function (compare Suppl. 2, Table S2), confirming that the scaling between size and weight is linear within the studied size range.

Having established that the slope of a linear regression can be used to describe the size–weight relationships within samples, we can investigate differences among samples and species. First, we note that variation within species is smaller than differences among species (Fig. 7a). The size–weight slope of *G. bulloides* appears consistently much smaller than that of *G. ruber* (white) and *G. elongatus*, whereas the values for the two *Globigerinoides* species are similar. Neither

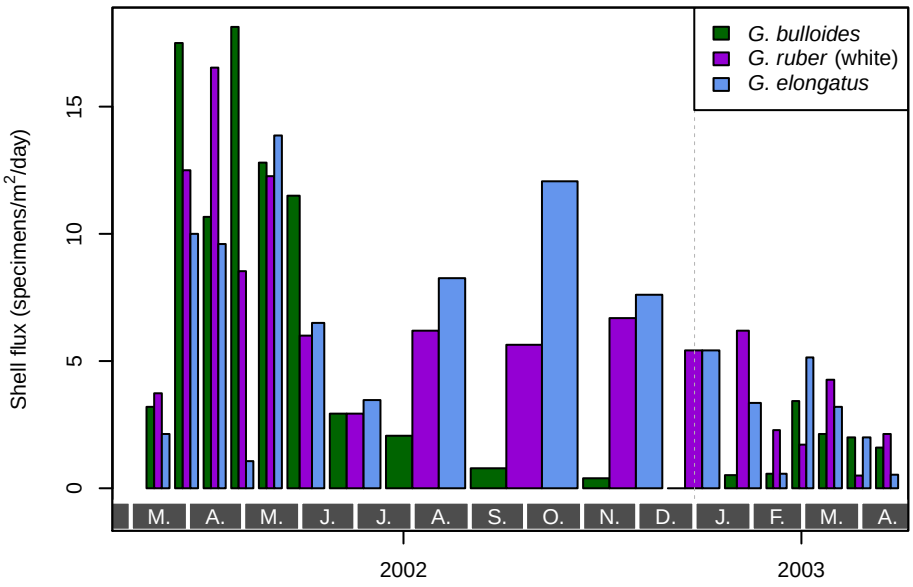



**Figure 4.** Flux of *Globigerina bulloides*, *Globigerinoides ruber* (white), and *Globigerinoides elongatus* sampled from March 2002 until April 2003 with sediment trap L1/K276. The flux was calculated on the basis of counted absolute abundances in the size fraction $> 150\,\mu m$, the trap opening of $0.5\,m^2$, and the sampling duration of each sample (Suppl. S1). Grey boxes with letters at the bottom indicate months, the vertical, dashed, grey line marks the end of 2002.

could we detect a difference in the slope values between *G. ruber* (white) and *G. elongatus*, nor were the pooled *Globigerinoides* values significantly different from those of either species (Table 1). Second, we examine the temporal evolution of the size–weight slope values for all three species (Fig. 8). This plot reveals that only in one sample for *G. bulloides* does the slope deviate significantly from the average value for the species. The significance is here assessed by finding a hypothetical value of the size–weight slope that falls within the $95\,\%$ confidence interval of as many samples as possible. Because the majority of the slope values for each species do not deviate significantly from each other, it seems that the size–weight scaling within each species did not change throughout the studied period.

Because the size–weight scaling seems constant through time for each species and the distribution of $AD_i$ values within nearly all samples are unimodal (Suppl. 2, Table S1), it is possible to use the average AD of all specimens within a sample as a robust estimate of calcification intensity. This is equivalent to comparing the intercepts of the size–weight regression lines, assuming their slopes are the same. The resulting values represent a reliable form of size-normalized weight. The temporal evolution of calcification intensity in all three species is shown in Fig. 9. This reveals that calcification intensity of *G. ruber* (white) and especially *G. elongatus* seems to be lower during late winter and the highest values are reached during June and July. In contrast, calcification intensity of *G. bulloides* appears to be rather constant throughout the year. In all samples, the calcification intensity of *G. elongatus* is generally larger than that of *G. ruber* (white) and the values for *G. bulloides* are consistently the smallest (Fig. 7b, Table 2).

**Table 1.** Pairwise comparison (Mann–Whitney $U$ test with $p$-values adjusted after Benjamini and Hochberg (1995)) of the size–weight scaling slope of *Globigerinoides ruber* (white), *Globigerinoides elongatus*, both species of *Globigerinoides* pooled together, and *Globigerina bulloides* sampled from March 2002 until April 2003 with sediment trap L1/K276. *Globigerina bulloides* shows a significantly different size–weight scaling than *Globigerinoides*, but the scaling of *G. ruber* (white) and *G. elongatus* are indistinguishable (compare Fig. 7a).

| Species 1 | Species 2 | adj. $p$-value |
|---|---|---|
| *Globigerinoides* | *G. bulloides* | $< .001$ |
| *Globigerinoides* | *G. ruber* (white) | .493 |
| *Globigerinoides* | *G. elongatus* | .348 |
| *G. ruber* (white) | *G. elongatus* | .337 |
| *G. ruber* (white) | *G. bulloides* | $< .001$ |
| *G. elongatus* | *G. bulloides* | $< .001$ |

## 4 Discussion

### 4.1 Scaling of size and weight among individual shells

All methods used to quantify calcification intensity in planktonic Foraminifera normalize weight to a measure of shell

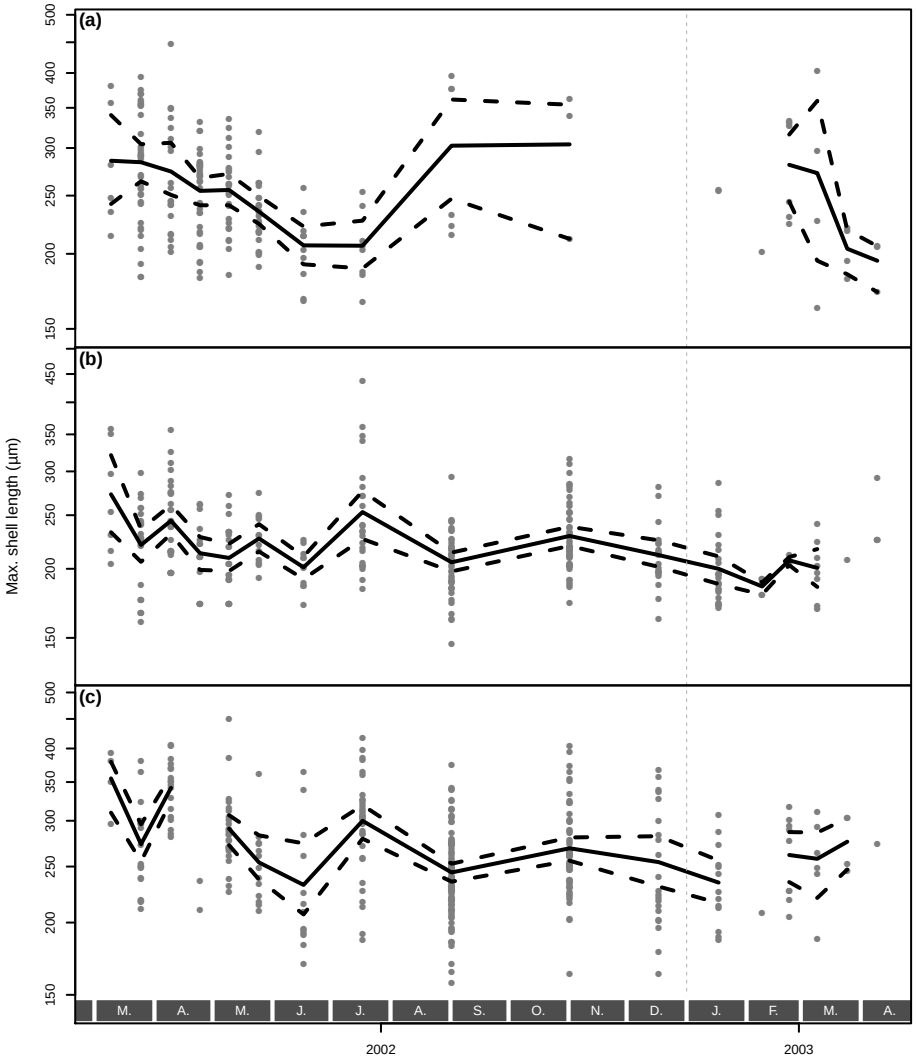



**Figure 5.** Shell sizes of *Globigerina bulloides* (a), *Globigerinoides ruber* (white) (b), and *Globigerinoides elongatus* (c) sampled from March 2002 until April 2003 with sediment trap L1/K276. Raw size values (expressed as Feret diameter) are indicated as symbols. The mean size per species per sample (solid lines) and its bootstrapped 95 % confidence interval (dashed lines) is also shown. Grey boxes with letters at the bottom indicate months, the vertical, dashed, grey line marks the end of 2002. Note the log-scaling of the $y$-axis.

size. This concept is based on the assumption that the scaling of size and weight is consistent within the analysed size range. Alternatively, if the scaling between size and weight varied with size, differences in calcification intensity determined in a given size fraction could exist for two reasons (Fig. 1). First, an observed offset could reflect an offset in the scaling line, indicating that the shells in one of the populations were more heavily calcified irrespective of size. Second, the same difference could reflect a change in the slope of the scaling line, implying that in one of the populations compared to the other population, larger shells were more heavily calcified than smaller shells. These alternatives would imply fundamentally different processes responsible for the same amount of observed change in calcification intensity, when expressed as some form of size-normalized weight.

Theoretically, because the analysed planktonic Foraminifera belong to the same spinose clade, have the same general shell morphology, and build their shells in a similar way, there should be no a priori reason why the scaling slopes between shell size and weight should be different. In this way, the assumption of the classical methods to quantify calcification intensity appears justified. Indeed our analysis reveals that the scaling, when expressed as volume to weight relationship, is consistent within each species and does not change for populations exposed to the contrasting summer or winter conditions (Fig. 8). However, we observe statistically significant, consistently large dif-

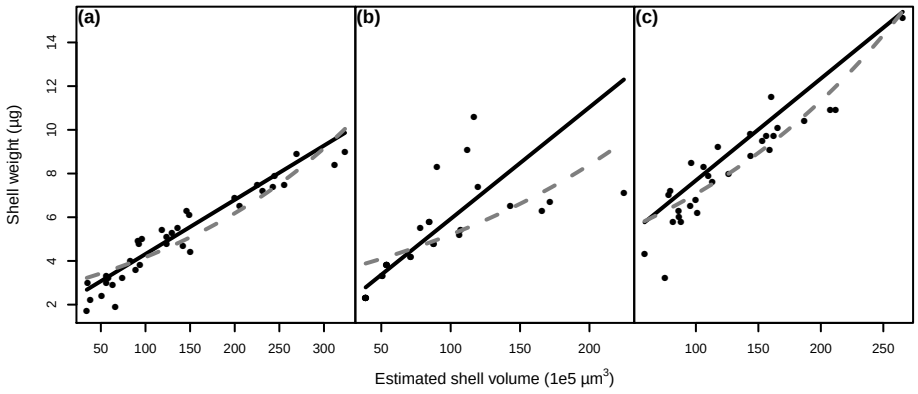



**Figure 6.** Calculation of the size–weight scaling per sample exemplarily shown in the richest sample each of *Globigerina bulloides* (a, Sample 3), *Globigerinoides ruber* (white) (b, Sample 4), and *Globigerinoides elongatus* (c, Sample 10), sampled from March 2002 until April 2003 with sediment trap L1/K276. The measured cross-sectional area of the shell was recalculated to the approximated shell volume, and its relationship with the associated shell weight was tested using a Kendall–Theil robust line fitting (solid black lines). The slope of the regression line corresponds to the size–weight scaling plotted in Fig. 8. The best fitting exponential function through the points is also plotted as dashed grey line in each case, but in the example it would only in the case of *G. elongatus* significantly increase the fit of the model.

**Table 2.** Pairwise comparison (Mann–Whitney $U$ test with $p$-values adjusted after Benjamini and Hochberg (1995)) of the calcification intensity (expressed as area density) of *Globigerinoides ruber* (white), *Globigerinoides elongatus*, both species of *Globigerinoides* pooled together, and *Globigerina bulloides* sampled from March 2002 until April 2003 with sediment trap L1/K276. The calcification intensity is different between all species, and most importantly the calcification intensity of the pooled *Globigerinoides* is not representative for either of the two species pooled together (compare Fig. 7b).

| Species 1 | Species 2 | adj. $p$-value |
|---|---|---|
| *Globigerinoides* | *G. bulloides* | $< .001$ |
| *Globigerinoides* | *G. ruber* (white) | $< .001$ |
| *Globigerinoides* | *G. elongatus* | $< .001$ |
| *G. ruber* (white) | *G. elongatus* | $< .001$ |
| *G. ruber* (white) | *G. bulloides* | $< .001$ |
| *G. elongatus* | *G. bulloides* | $< .001$ |

ferences in the scaling slope between *Globigerina bulloides* and both *Globigerinoides* species (Fig. 7a). This means that there is not a universal scaling slope between size and weight among planktonic Foraminifera. The size–weight relationships in the adult specimens analysed in this study must be the result of different ontogenetic calcification trajectories. If we consider that all planktonic Foraminifera commence calcification as geometrically similar prolocular stages (Brummer and Kroon, 1988, part I), then different size–weight scaling slopes among species in their adult stage require different ontogenetic trajectories in this scaling. The differences in scaling imply that absolute values of the size-normalized weight (irrespective of its precise formulation) are not comparable among species. Although we only observe differences in the scaling slope between species, it cannot be excluded that such differences also occur within species.

The lack of significant temporal variation in the scaling slope between size and weight within individual species may reflect the large confidence intervals on the slope, which are for obvious reasons mainly a function of sample size. Therefore, we ventured to investigate whether the observed variation in slope within species correlates with any of the candidate environmental parameters: temperature, as main factor influencing the pace of cellular processes, and chlorophyll a concentration as an indicator of productivity and nutrient availability (Fig. 10). Given that the calcification intensity of foraminiferal shells is itself considered to be driven by one or more of those environmental parameters, a correlation of the size–weight scaling with the same environmental parameters would introduce a cross-correlation that would render the calcification intensity prone to misinterpretation. A robust multiple linear regression of the scaling slope values indicates no significance of either environmental parameter in any of the species investigated (Table 3), however. While this does not rule out the environmental forcing of the scaling slope with any as yet untested environmental factor, this analysis seems to support the observation that the scaling slope may be an intrinsic characteristic of each species, which is invariant to environmental perturbations. If this conclusion holds, then size-normalized weight can be used as a proxy for calcification intensity within species.

**Table 3.** Robust multiple linear regression $p$-values for the influence of environmental parameters on the size–weight scaling of *Globigerinoides ruber* (white) and *Globigerinoides elongatus*, both *Globigerinoides* species combined, and *Globigerina bulloides* sampled from March 2002 until April 2003 with sediment trap L1/K276. Neither chlorophyll a content nor temperature (SST) had an influence on the scaling slopes in any species.

| | *G. bulloides* | *G. ruber* (white) | *G. elongatus* | *Globigerinoides* |
|---|---|---|---|---|
| Intercept | .022 | .934 | .023 | .729 |
| Chlorophyll a | .155 | .967 | .073 | .836 |
| SST | .144 | .970 | .141 | .860 |

### 4.2 Measuring calcification intensity

Our analyses of the size–weight scaling have shown that calcification intensity within species can be approximated by size-normalized weight. Because of the availability of single-shell measurements, we can here use and analyse the distribution of area density values as applied by Marshall et al. (2013). This approach is exact, but time-consuming. Therefore we test how the mean AD values would differ from a mean area density (MAD) determined such that the size of the specimens is measured individually, but all specimens from a single sample are weighed together (compare Weinkauf et al., 2013). Confidence intervals for the MAD can be estimated by repeated determination of the MAD in random subsamples of one sample, and bootstrapping confidence intervals for variable sample sizes on the basis of that dataset (Weinkauf et al., 2013). Here we used our data to simulate MAD values for all three species and compared them with the corresponding mean of the individual $\mathrm{AD}_i$ values per sample (Suppl. 2, Fig. S2a). We found, that both values are highly and significantly correlated, with the slope of the regression line in no case being significantly different from one. We can thus conclude that the more efficient method of MAD is likely to yield comparative results to the more time-consuming determination of individual $\mathrm{AD}_i$ values. However, when comparing the bootstrapped confidence interval for MAD calculated as in Weinkauf et al. (2013) with the confidence interval determined from the distribution of the individual $\mathrm{AD}_i$ values, a significant mismatch is observed. The bootstrap procedure of Weinkauf et al. (2013) tends to underestimate the uncertainty of the MAD values on average by approximately a factor of two (Suppl. 2, Fig. S2b). Thus, whilst the MAD approach seems to yield reliable mean values, the associated uncertainty seems difficult to estimate. As shown previously, both AD and MAD methods are likely to be much superior to the sieve-based approaches, especially to the unqualified sieve-based weight (Beer et al., 2010a).

An interpretation of calcification intensity measured in this way requires that AD and MAD are independent of the size–weight scaling. While there is no statistically significant systematic change of this scaling in the course of a year or in relation to the environment, there remains a certain variability within species that appears unexplained (compare Figs 7 and 8). Should this variability be systematically linked to calcification intensity, then the observed calcification intensity could at least partly reflect changes in the size–weight scaling, and the resulting difference in calcification intensity would be difficult to interpret. To exclude this possibility, we tested for such a relationship in all three species by applying a Kendall–Theil robust line fitting to the calcification intensity in dependence of the size–weight scaling slope (Fig. 11). The relationship was not significant in any of the three species, indicating a complete independence of the variability in both values and supporting the interpretation that the observed variability in the size–weight scaling slope within species is stochastic.

### 4.3 Determinants of calcification intensity within species

Considering the previous analyses, we may now use the temporal distribution of area density values of all specimens within one species to search for potential controlling environmental parameters. Candidate parameters are temperature and chlorophyll a concentration. We disregard salinity because it is highly correlated with temperature (Fig. 10) and could only influence calcification by influencing the carbonate system, which is (1) nearly constant during the sampling period and (2) only to a minor degree driven by salinity. To this end, we fitted a series of GLMs (based on the gamma distribution, with identity as link function), and compared their explanatory value. A GLM analyses the reaction in one dependent variable (here: calcification intensity) to several independent variables at the same time. GLMs of increasing complexity incorporate the potential influence of increasingly more environmental parameters, and thus necessarily explain a higher degree of the observed variance, reducing the amount of unexplained variance $\epsilon$. The Akaike information criterion $\mathrm{AIC}_c$ can then be used to infer, whether the increase in explanatory value of a more complex model is worth the higher complexity of that model.

The greatest problem in such an analysis is the often occurring multicollinearity between environmental parameters. To that end, we performed a Pearson product-moment correlation between temperature and productivity. Following the suggestion of Dormann et al. (2013) to consider a significant correlation with a correlation coefficient of $> 0.7$ as a sign

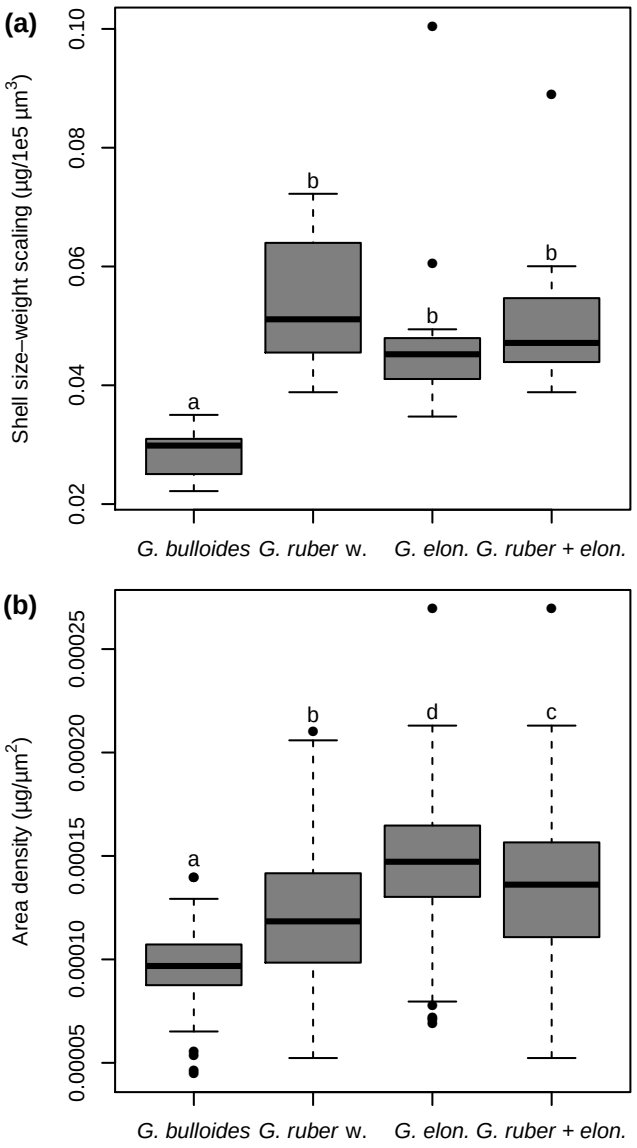



**Figure 7.** Size–weight scaling (a) and calcification intensity (b, expressed as area density) of *Globigerinoides ruber* (white), *Globigerinoides elongatus*, both *Globigerinoides* species pooled together, and *Globigerina bulloides* sampled from March 2002 until April 2003 with sediment trap L1/K276. Boxplots show the median (thick line), interquartile range (grey box), $1.5\times$ interquartile range (whiskers), and outliers (black dots). The group assignment according to pairwise comparisons (compare Tables 1 and 2) is indicated by lower case letters above the whiskers.

for significant collinearity, we observe significant collinearity between temperature and chlorophyll a ($r = -0.828$, $p < .001$). However, because of the fact that high collinearities in a GLM increase the chance of a type II error, thus making it less likely to detect a significant relationship, without increasing the false-positive rate (Dormann et al., 2013), we conclude that we can perform the intended analyses on our data, but realize that the statistical tests are likely to be too conservative. For all GLMs we tested the significance of residual deviance, which tests if the model explains the data well, or if predicted values and residuals are correlated (which would indicate a biased analysis). The residual deviance was insignificant ($p = 1$) for all our analyses, indicating an unbiased analysis.

We begin with an analysis including both species of *Globigerinoides*. The most simple Model (1) pools both species of *Globigerinoides* and includes as candidate controlling variables the SST ($T$) and chlorophyll a content ($Ca$). While *Globigerinoides* (as a symbiont bearing genus) may by itself be less dependent on productivity, favourable conditions for the phytoplankton might also indicate favourable conditions for the foraminiferal symbionts, which is why productivity has been considered in the model. Some authors (de Villiers, 2004; Gonzalez-Mora et al., 2008; Naik et al., 2011, 2013) suggested that calcification intensity of foraminiferal shells reflects growth under optimal environmental conditions. Assuming that the suitability of an environment directly influences the abundance of the species, a more complex Model (2) thus assumes that the AD also changes as a function of the flux ($F$) of the species. Next, Model (3) assumes that the different though closely related species (*Bs*) *G. ruber* (white) and *G. elongatus* may calcify differently under all conditions. Finally, Model (4) expands this hypothesis by assuming that different species may also show different reactions in their shell calcification towards changes in the environmental parameters.

$$\mathrm{AD} = x_1 T + x_2 Ca + \epsilon \quad (1)$$

$$\mathrm{AD} = x_1 T + x_2 Ca + x_3 F + \epsilon \quad (2)$$

$$\mathrm{AD} = x_1 T + x_2 Ca + x_3 F + x_4 Bs + \epsilon \quad (3)$$

$$\begin{aligned}\mathrm{AD} = {} & x_1 T + x_2 Ca + x_3 F + x_4 Bs \\ & + x_4 Bs \times x_1 T + x_4 Bs \times x_2 Ca \\ & + x_4 Bs \times x_3 F + \epsilon\end{aligned} \quad (4)$$

The analysis shows that Model (4) explains the data best. Following the Akaike information criterion test, Model (3) ($\Delta\mathrm{AIC}_c = 16.5$), Model (2) ($\Delta\mathrm{AIC}_c = 57.3$), and Model (1) ($\Delta\mathrm{AIC}_c = 57.7$) were all clearly inferior.

An examination of the coefficients of Model (4) allows an assessment of factors that most influence the calcification intensity within the two *Globigerinoides* species. This reveals that the pooled *Globigerinoides* calcification is mainly driven by SST and chlorophyll a, with higher calcification intensities observed during times of raised SST and lower productivity (Fig. 12, Table 4). Both species show a constant offset in calcification intensity, confirming results already discussed above, as well as a significant interaction term between species, and SST and chlorophyll a, implying that

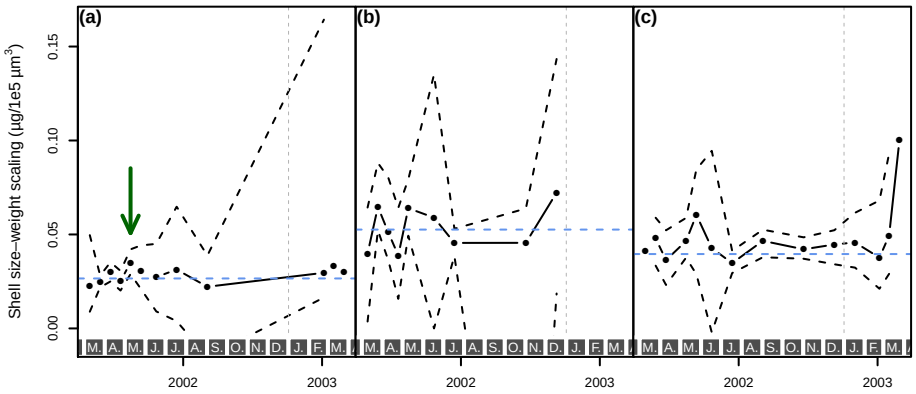



**Figure 8.** Estimated size–weight scaling (solid lines with dots) and its 95 % confidence interval (dashed lines) of *Globigerina bulloides* (a), *Globigerinoides ruber* (white) (b), and *Globigerinoides elongatus* (c), sampled from March 2002 until April 2003 with sediment trap L1/K276. Some samples either contained no or too few specimens to estimate the scaling or its confidence interval, or were not significant in the Kendall–Theil robust line fitting, and were thus not included in the analysis. The horizontal, dashed blue line indicates a possible annual mean value of the size–weight scaling per species, that would never fall outside the 95 % confidence interval (in *Globigerina bulloides* this only works if one sample from early May 2002, marked by the green arrow, is regarded as an outlier). Grey boxes with letters at the bottom indicate months, the vertical, dashed, grey line marks the end of 2002.

calcification in *G. ruber* (white) and *G. elongatus* respond differently to changes in temperature and productivity. Shell flux (as indicator of the suitability of the environment) does not seem to affect shell calcification in either species, but the interaction term between species and flux is significant. When applying a Kendall–Theil robust line fitting solely on the correlation between flux and AD (i.e. disregarding other factors) one indeed finds a significant positive correlation ($p < .001$, $R^2 = 0.459$) between abundance and shell calcification in *G. elongatus*, but not in *G. ruber* (white) ($p < .564$, $R^2 = -0.006$) (Suppl. 2, Fig. S1). We must therefore conclude that calcification intensity is indeed influenced by the suitability of the environment in some but not all species.

Until now, there has been little evidence for an influence of temperature on calcification intensity. Only Marshall et al. (2013, fig. 4) presented a significant relationship between area density and SST, but they attributed it to a collinearity of SST with $CO_3^{2-}$, favouring carbonate chemistry as the main explanatory variable. Raised temperature leads to higher metabolic rates as well as higher carbonate saturation, thus favouring calcification both abiotically and biotically, providing excess energy for biomineralisation. The latter only applies as long as the temperature increase does not exceed the physiological optimum of a species, however. Since the carbonate system has likely been rather stable during the time interval investigated here, our data imply that the biotic component may play a major role in shell calcification intensity, regardless of (or maybe despite) changes in $CO_3^{2-}$.

The evidence for a relationship between calcification intensity and optimum growth conditions in *G. elongatus* seems to be in support of an existence of energy trade-offs between calcification and growth under suboptimum conditions, as postulated by de Villiers (2004) and Beer et al. (2010b). On the other hand, the observed negative relationship of calcification intensity with productivity contradicts the idea that higher nutrient content of the surface water might indicate favourable conditions for the symbionts or that food availability alone may free more energy for calcification. Instead, we hypothesize that higher surface water productivity may lead to more light attenuation, so that under bloom conditions the foraminiferal symbionts receive less light, and calcification intensity is reduced, as observed in laboratory experiments manipulating the light levels in the

**Table 4.** Results of the most informative generalized linear model (Model 4) for the calcification intensity of *Globigerinoides* species from trap L1/K276 in the North Atlantic. The model implies that calcification is mainly driven by temperature and surface water productivity, but not by environmental suitability as indicated by shell flux. It further confirms the results of the Mann–Whitney $U$ test that the two biospecies *G. ruber* (white) and *G. elongatus* differ in base calcification intensity (compare Fig. 7b, Table 2), but further implies that they also show different reaction terms to virtually all environmental parameters.

| | Standard error | $t$-value | $p$-value |
|---|---|---|---|
| Intercept | $5.166 \times 10^{-5}$ | $-5.042$ | $< .001$ |
| SST | $2.379 \times 10^{-6}$ | 7.373 | $< .001$ |
| Chl. a | $5.209 \times 10^{-5}$ | 4.579 | $< .001$ |
| Shell flux | $4.954 \times 10^{-7}$ | 0.902 | .368 |
| Biospecies | $7.122 \times 10^{-5}$ | 4.524 | $< .001$ |
| SST × biospecies | $3.198 \times 10^{-6}$ | $-4.467$ | $< .001$ |
| Chl. a × biospecies | $7.934 \times 10^{-5}$ | $-3.058$ | .002 |
| Shell flux × biospecies | $8.277 \times 10^{-7}$ | 2.196 | .029 |

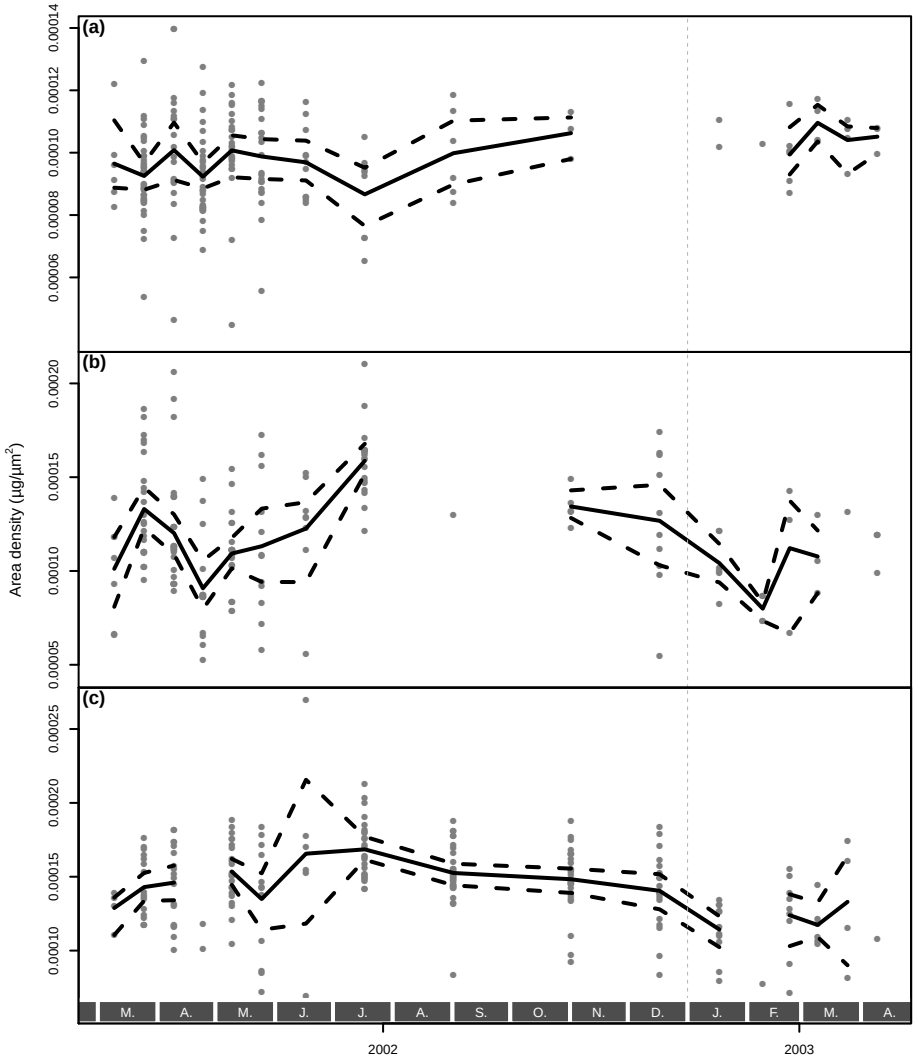



**Figure 9.** Calcification intensities (expressed as area density) of shells of *Globigerina bulloides* (a), *Globigerinoides ruber* (white) (b), and *Globigerinoides elongatus* (c) sampled from March 2002 until April 2003 with sediment trap L1/K276. Raw values (grey dots) are plotted together with the mean value (solid lines) and its bootstrapped 95 % confidence interval (dashed lines) per sample. Grey boxes with letters at the bottom indicate months, the vertical, dashed, grey line marks the end of 2002.

symbiont-bearing species *Globigerinoides sacculifer* (Caron et al., 1982).

The discovery of different base calcification intensities and different reactions of calcification intensity to environmental variables between the two closely related species *G. ruber* (white) and *G. elongatus* is most interesting. Merging these species in analyses of calcification intensity would introduce an environmental interaction term that cannot be controlled. As we will discuss below, patterns of calcification observed in past studies potentially lumping these forms under the same category may be severely affected by this interaction.

The general validity of the inferences based on the analyses of calcification intensity in *Globigerinoides* can be assessed by replicating the same analytical framework for calcification intensity data based on the species *G. bulloides*. For this species, only Models (1) and (2) can be considered because all data are derived from the same species. Replicating the analysis as carried out for *Globigerinoides*, we find for *G. bulloides* Model (2) to be the most informative, with Model (1) being clearly distinguishable and inferior ($\Delta\mathrm{AIC}_c = 5.3$). The model indicates that there is no influence of either SST or chlorophyll a on calcification intensity in this species (Fig. 13a, Table 5). The lack of reaction of shell calcification in *G. bulloides* towards SST could indicate that the temperature effect in *Globigerinoides* may be mediated by its symbionts, which would also be consistent with *G. bulloides* lack of reaction to chlorophyll a concentration.

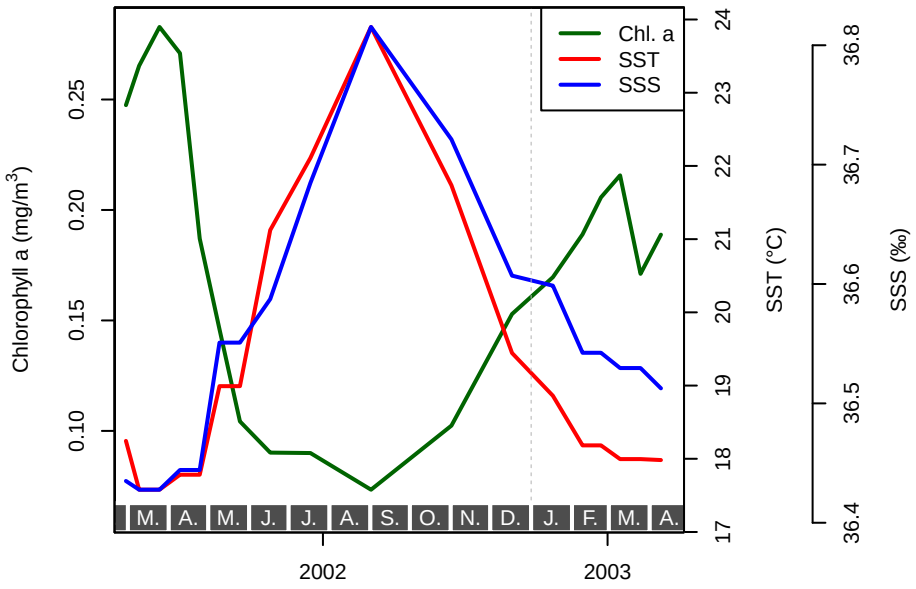



**Figure 10.** Mean values of chlorophyll a content of the surface waters (Yoder and Kennelly, 2005), sea surface temperature (SST), and sea surface salinity (SSS) (http://climexp.knmi.nl/) for the sampling period in the catchment area of sediment trap L1/K276. Since salinity is highly correlated with temperature and affects the carbonate saturation state only by a factor of ten less than temperature, we did not include it in our analyses for the environmental forcing of shell calcification.

**Table 5.** Results of the most informative generalized linear model (Model 2) for the calcification intensity of *Globigerina bulloides* from trap L1/K276 in the North Atlantic. The model implies that calcification intensity in that species is influenced by environmental suitability, but is otherwise robust against environmental change.

| | Standard error | $t$-value | $p$-value |
|---|---|---|---|
| Intercept | $2.812 \times 10^{-5}$ | 5.342 | $< .001$ |
| SST | $1.260 \times 10^{-6}$ | $-1.772$ | .078 |
| Chl. a | $2.310 \times 10^{-5}$ | 1.014 | .312 |
| Shell flux | $2.274 \times 10^{-7}$ | $-2.788$ | .006 |

Model (2) further indicates a significant correlation between flux and calcification in *G. bulloides* (which is also supported by a linear regression, compare Suppl. 2, Fig. S1a). In contrast to predictions by de Villiers (2004) and Naik et al. (2013), however, this correlation is negative, i.e. higher abundances in *G. bulloides* are correlated with less calcified shells. This puzzling observation can be explained in two ways. First, if high abundance in the species reflects faster growth, there is less time available for biomineralisation and as a result the shells are less calcified than in less suitable environments. Alternatively, *G. bulloides* is known to harbour significant genetic diversity and the studied region is likely inhabited by as many as three different genetic lineages within the morphospecies (Darling and Wade, 2008). If, like in *Globigerinoides*, these cryptic lineages have a species-specific response of calcification intensity to environmental parameters, the resulting 'pooled' signal could be entirely confounded by this effect.

### 4.4 Species-specific calcification patterns

Our results indicate that the response of calcification intensity to environmental parameters is species-specific. This applies not only to the existence of significant relations, but also to its sign. In addition, we have for the first time provided evidence that not only the calcification intensity, but also the size–weight scaling differs among species (Fig. 7a). Although studies on the determinants of calcification intensity (shell weight) in planktonic Foraminifera have been conducted using different material and different methodology, there now exist enough data to attempt a comparison of the types of responses implied by individual studies (Table 6). Beside the fact that this study is one of the few investigating the effects of productivity on calcification, our results are broadly coherent with the patterns documented in earlier studies. The negative correlation between flux and calcification intensity in *G. bulloides* has been also observed by Aldridge et al. (2012) in plankton samples from the North Atlantic. The fact that this counter-intuitive result has now been replicated may indicate that it hints at the existence of a poorly understood aspect of the environmental control of calcification in that species. It may still be an effect of combining different genetic lineages of this species in the analysis, but we note that in our study and that by Aldridge et al.

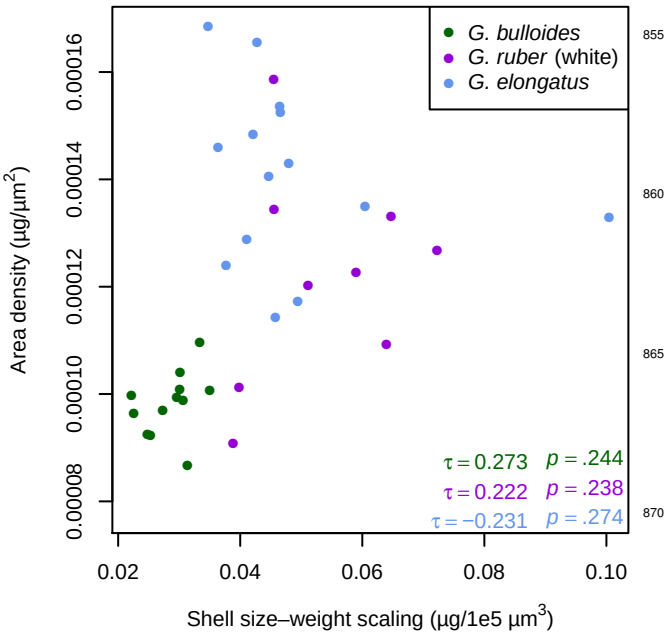



**Figure 11.** Calcification intensity (expressed as area density) as function of the size–weight scaling slope in *Globigerina bulloides*, *Globigerinoides ruber* (white), and *Globigerinoides elongatus* sampled from March 2002 until April 2003 with sediment trap L1/K276. The correlation coefficient $\tau$ and the $p$-value for a dip of the regression line being significantly different from zero are provided. No significant relationship between the two parameters could be detected in any species.

(2012), the genetic lineages that may have been pooled are likely to be different.

The meta-analysis presented in Table 6 reveals that higher temperature and higher carbonate saturation, either determined directly or inferred from indirect proxies, seems to generally favour calcification. The effect of temperature is less frequently observed and confounded by collinearity (Marshall et al., 2013) and potentially even interaction with carbonate saturation (Manno et al., 2012). On the other hand, the effect of productivity and optimal growth conditions is ambiguous. Interestingly, these results seem to apply to symbiont-bearing and asymbiotic species alike, which would indicate that the presence of symbionts may affect the absolute values of calcification intensity and size–weight slope, but it does not modify the sign of the response of calcification intensity to the main candidate environmental parameters.

The existence of species-specific response types and offsets in calcification intensity and size–weight scaling implies a potentially high sensitivity of the observed response type and strength to the accuracy of species identification. Our data provide first evidence for distinct patterns recorded by *G. ruber* (white) and *G. elongatus*. Traditionally, these species were often deliberately or unintentionally pooled for various purposes, assuming that *G. ruber* in its broad definition introduced by Parker (1962) represent one biological species. In reality, as shown already by isotopic and trace-element analyses on *G. ruber* morphotypes (e.g. Steinke et al., 2005), the two forms lumped within the broad concept of *G. ruber* (white) represent genetically distinct lineages with different ecological preferences (Aurahs et al., 2011). Using the data in our study, we can simulate the effect of pooling *G. ruber* (white) and *G. elongatus* on calcification patterns in such a synthetic taxon. We observe that in the pooled dataset, already the first assumption needed to meaningfully interpret calcification intensity as approximated by AD is violated. In the pooled dataset, the size–weight scaling is not consistent across samples, giving the impression of the existence of steeper scaling slopes in winter (Fig.14). In reality, this simply reflects the times when *G. ruber* (white) was more abundant than *G. elongatus* (Fig. 4). As a result, the temporal evolution of calcification intensity in a pooled *Globigerinoides* dataset (Fig.14) reveals spurious patterns that are not observed in the data for either species and reflect an induced negative correlation between the scaling slope and calcification intensity in the pooled data. These results imply that combining *G. ruber* (white) and *G. elongatus* into a single morphospecies for analyses of calcification intensities (e.g. trap or plankton tow series) introduces an error that can neither be eliminated nor objectively quantified. Several of the investigations summarised in Table 6 may indeed be affected by this issue, explaining the lack of sensitivity of pooled *G. ruber* calcification data to temperature and carbonate ion as observed by Naik et al. (2013) as well as the results by Beer et al. (2010b), which otherwise remains the only case documenting a negative correlation between calcification intensity and carbonate saturation in planktonic Foraminifera.

### 4.5 Optimum growth conditions reflected by shell size distribution

The concept of growth under optimum conditions in planktonic Foraminifera has been originally devised to explain patterns of shell size distribution in surface sediments. This concept (Hecht, 1976; Schmidt et al., 2003, 2004) is based on the observations that largest mean shell size occurs in samples where the analysed species meet their environmental optimum. Since shell size data have been collected for the three species analysed in this study, it is possible to test independently the assumption of using species flux in the sediment trap series as approximation of optimum growth conditions. If the concept by Hecht (1976) holds, then shell size in *G. bulloides* should be largest during the late winter and spring when its abundance is highest and temperatures are lowest, closer to the optimum of the species, whose highest abundance in the sediment is to the north of the sediment trap region (Fig 2b). For *G. ruber* (white) and *G. elongatus* the test will be more difficult because the preferences of the two species are not known from sediment distribution, be-

**Table 6.** Review of environmental parameters that have been found to influence foraminiferal shell calcification in several studies thus far, including the species and type of material used. + = positive correlation, − = negative correlation, 0 = no influence detected

| Symbionts | Species | Publication | Material type | Temperature | Temperature range (°C) | Productivity | Carbonate ion | $[CO_3^{2-}]$ range ($\mu mol\ kg^{-1}$) | Optimal growth conditions | other |
|---|---|---|---|---|---|---|---|---|---|---|
| + | *G. ruber*[d] | Davis et al. (2013) | Fossil sediment | 0 | max. 7 | | | | | |
| + | *G. ruber* (white)[c] | Gonzalez-Mora et al. (2008) | Fossil sediment | +[a] | 9 | | +*[a] | NA | 0[a] | |
| | | Beer et al. (2010b) | Plankton net samples | | | | − | 60 | 0 | |
| | | Naik et al. (2011) | Fossil sediment | 0 | 4 | | 0* | NA | + | |
| + | *G. ruber* (white) + *G. elongatus* | de Moel et al. (2009) | Surface sediment | | | | +* | max. 18 | | influence of monsoon seasonality |
| | | Naik et al. (2013) | Trap samples | 0 | max. 4 | | 0* | NA | + | |
| + | *G. ruber* (white) | This study | Trap samples | + | 7 | − | 0* | 7 | 0 | |
| + | *G. ruber* (pink) | Marshall et al. (2013) | Trap samples | +[b] | 6 | | + | 70 | | − with phosphate[b] |
| | | Weinkauf et al. (2013) | Fossil sediment | | | | +* | NA | 0 | |
| + | *G. elongatus* | This study | Trap samples | + | 7 | − | 0* | 7 | + | |
| + | *G. sacculifer* | Broecker and Clark (2001a) | Surface sediment | | | | + | 60 | | |
| | | Lombard et al. (2010) | Culture experiments | | | | + | 495 | | + with light irradience |
| | | Naik et al. (2010) | Fossil sediment | −[b] | NA | | +* | 45 | | |
| | | Marshall et al. (2013) | Trap samples | +[b] | 5 | | + | 90 | | |
| + | *N. dutertrei* | Broecker and Clark (2001a) | Surface sediment | | | | + | 60 | | |
| + | *O. universa* | Spero et al. (1997) | Culture experiments | | | | + | max. 700 | | |
| | | Bijma et al. (1999) | Culture experiments | | | | + | 800 | | |
| | | Lombard et al. (2010) | Culture experiments | | | | + | 270 | | |
| | | Weinkauf et al. (2013) | Fossil sediment | | | | +* | NA | 0 | |
| − | *G. bulloides* | Barker and Elderfield (2002) | Surface and fossil sediment | 0[a] | 16 | | +* | 70 | | |
| | | de Villiers (2004) | Surface sediment | | | | | | +[a] | |
| | | Gonzalez-Mora et al. (2008) | Fossil sediment | +[a] | 8 | | +*[a] | NA | 0[a] | |
| | | Moy et al. (2009) | Trap samples and surface sediment | | | | +* | NA | | |
| | | Beer et al. (2010b) | Plankton net samples | | | | + | 120 | 0 | |
| | | Naik et al. (2011) | Fossil sediment | −[b] | 4 | | +* | NA | + | |
| | | Aldridge et al. (2012) | Plankton net samples | + | 3 | + | + | 33 | − | + with growth potential, − with phosphate and nitrate |
| | | Davis et al. (2013) | Fossil sediment | +[a] | 9 | | | | | |
| | | This study | Trap samples | 0 | 7 | 0 | 0* | 7 | − | |
| − | *G. inflata* | Barker and Elderfield (2002) | Surface and fossil sediment | 0[a] | 16 | | +*[a] | 70 | | |
| | | Weinkauf et al. (2013) | Fossil sediment | | | | +* | NA | 0 | |
| − | *G. puncticulata* | Davis et al. (2013) | Fossil sediment | +[a] | 9 | | | | | |
| − | *G. scitula* | Weinkauf et al. (2013) | Fossil sediment | | | | +* | NA | 0 | |
| − | *G. truncatulinoides* | Barker and Elderfield (2002) | Surface and fossil sediment | 0[a] | 12 | | +*[a] | 60 | | |
| | | de Villiers (2004) | Surface sediment | | | | | | +[a] | |
| − | *N. incompta* | Barker and Elderfield (2002) | Surface and fossil sediment | 0[a] | 16 | | +*[a] | 70 | | |
| | | de Villiers (2004) | Surface sediment | | | | | | +[a] | |
| | | Gonzalez-Mora et al. (2008) | Fossil sediment | 0[a] | 6 | | 0*[a] | NA | +[a] | |
| − | *N. pachyderma* | Manno et al. (2012) | Culture experiments | 0 | 5 | | + | 65 | | temperature moderates influence of carbonate ion |
| − | *P. obliquiloculata* | Broecker and Clark (2001a) | Surface sediment | | | | + | 60 | | |

*Carbonate ion concentration indirectly inferred; [a] Not explicitly tested, only implied; [b] Attributed to collinearity; [c] Unclear whether samples also included *G. elongatus* specimens; [d] Unclear whether samples included only *G. ruber* (white) or *G. ruber* (pink) or both

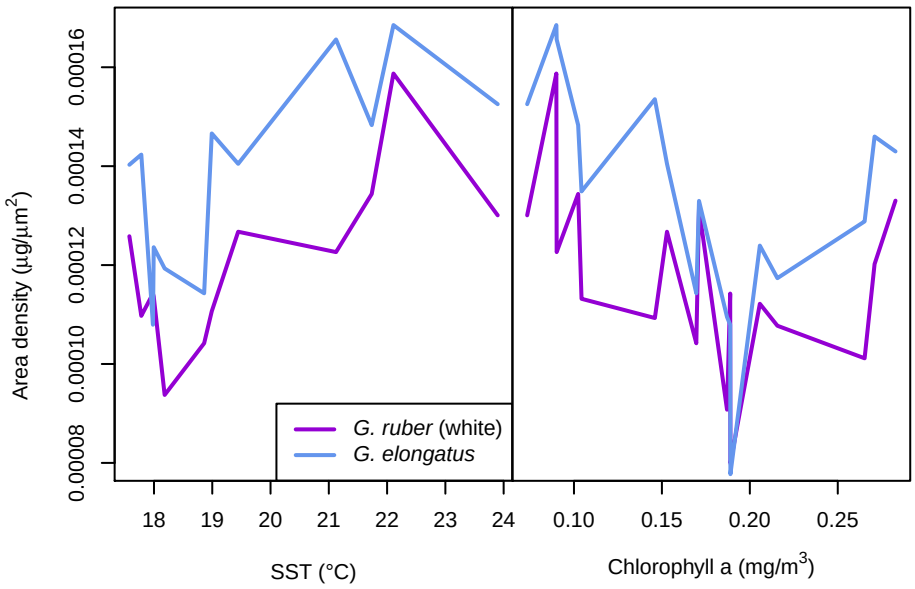



**Figure 12.** Interaction plot of the calcification intensity (expressed as area density, AD) in *Globigerinoides ruber* (white) and *Globigerinoides elongatus* sampled from March 2002 until April 2003 with sediment trap L1/K276. While both species generally show a positive correlation of AD with sea surface temperature (SST) and a negative correlation with surface water productivity, the offset between and strict non-parallelism of the lines shows that both species differ in base calcification intensity and react differently to changes in the environment (compare Table 4).

cause these two taxa nave not been separated (Fig 2c). For both species combined, sediment distribution data indicate a warmer optimum habitat than the average conditions at the sediment trap site, but peak abundance in the studied series occurs in summer and early spring, indicating that the abundance of those species may have reacted to factors other than temperature.

To test for correlation between environmental parameters and shell sizes in the three species analysed, we use the same GLM models as applied to the calcification data (Models (1)–(4)). In *G. bulloides* we find Models (1) and (2) to be indistinguishable ($\Delta\mathrm{AIC}_c = 1.1$). Both models indicate that shell sizes in this species are correlated with water temperature and productivity (Table 7). As expected from the optimum growth model, shell size of *G. bulloides* is negatively correlated with SST ($\rho = -0.142$) and positively correlated with productivity ($\rho = 0.306$), reflecting generally cooler and more productive optimum conditions of the species (Fig. 13b). A link of shell size with flux could not be observed however. This seems to reflect the asymmetry of flux between spring and autumn in 2002 and underlines the observation by Storz et al. (2009) of the large interannual variability at the studied site. It seems that in such situations the pattern of flux, rather than the absolute values, would be a better estimate of optimum growth conditions. For an analysis of the two species of *Globigerinoides* we find Model (4) to be the most informative, with the other models being clearly inferior (Model (3): $\Delta\mathrm{AIC}_c = 11.7$, Model (2): $\Delta\mathrm{AIC}_c = 160.1$, Model (1): $\Delta\mathrm{AIC}_c = 164.2$). However, this model implies that none of the environmental parameters nor flux has significantly affected the size distribution (Table 8), confirming the observation that shell sizes of both species remained rather similar throughout the studied period (Fig. 5).

**Table 7.** Results of the generalized linear Models (1) and (2) for the shell size of *Globigerina bulloides* from trap L1/K276 in the North Atlantic. The models show an influence of both sea surface temperature (SST) and productivity on the observed shell size.

| | Standard error | $t$-value | $p$-value |
|---|---|---|---|
| **Model 1** | | | |
| Intercept | 81.777 | 0.479 | .632 |
| SST | 3.723 | 2.128 | .035 |
| Chl. a | 82.481 | 4.533 | $< .001$ |
| **Model 2** | | | |
| Intercept | 96.957 | $-0.567$ | .571 |
| SST | 4.351 | 2.748 | .007 |
| Chl. a | 83.151 | 4.718 | $< .001$ |
| Shell flux | 0.762 | 1.787 | .076 |

The distribution of shell sizes in the three investigated species thus does not lend strong support to the hypothesis that optimum growth conditions result in largest shells sizes. Alternatively, it may be that absolute abundance as used in

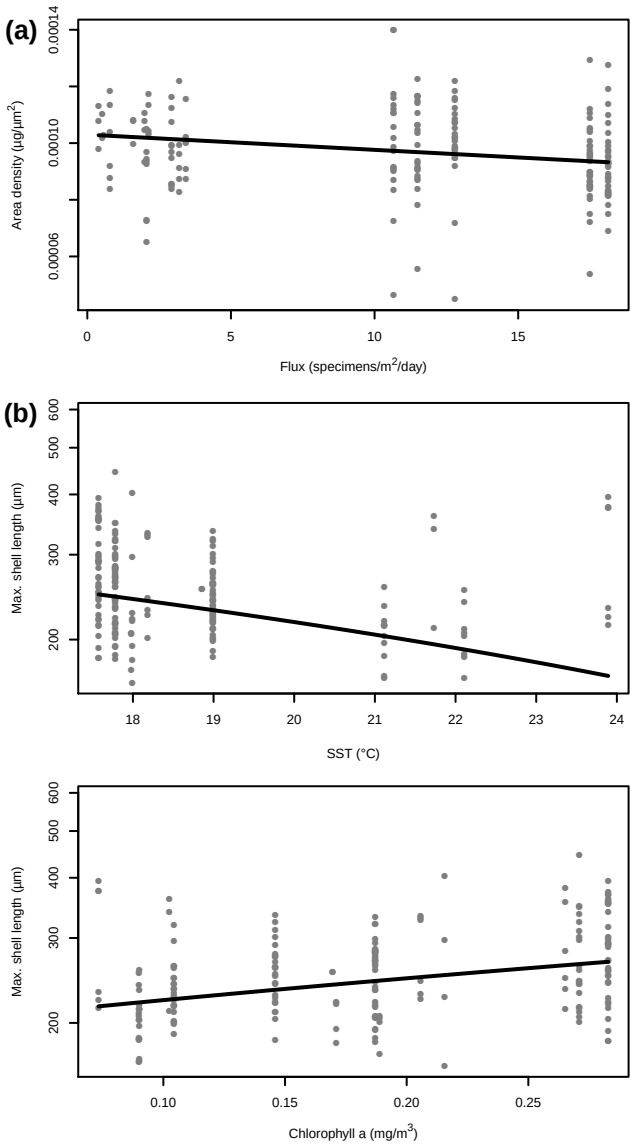



**Figure 13.** Cross plot of the calcification intensity (expressed as area density, AD) and shell size of *Globigerina bulloides* sampled from March 2002 until April 2003 with sediment trap L1/K276. Raw data values (grey dots) are plotted alongside the regression line from a Kendall–Theil robust line fitting. **(a)** The AD of shells of *G. bulloides* is decreasing when the species becomes more abundant (compare Table 5). **(b)** The shell size (Feret diameter) in *G. bulloides* shows a negative correlation with sea surface temperature (SST), but increases with productivity, likely due to the larger availability of nutrients and thus energy for the metabolism (compare Table 7). Note the log-scaling of the $y$-axis.

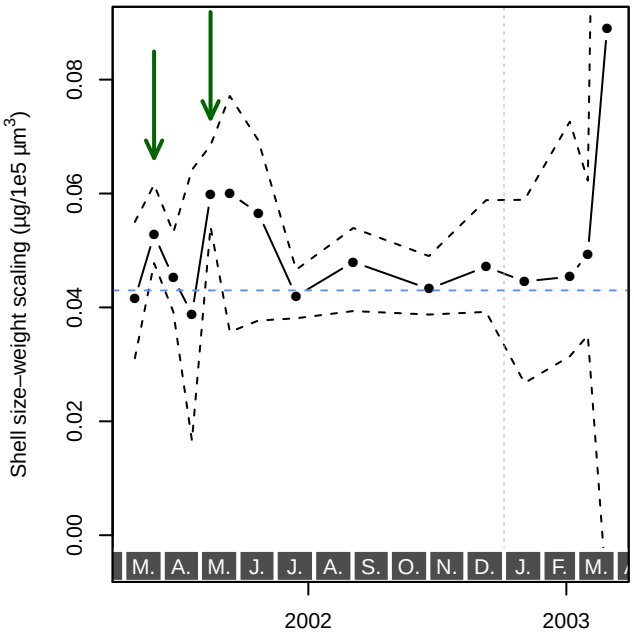



**Figure 14.** Estimated mean size–weight scaling (solid line with dots) and its 95 % confidence interval (dashed lines) of *Globigerinoides ruber* (white) and *Globigerinoides elongatus* sampled from March 2002 until April 2003 with sediment trap L1/K276 pooled together. Some samples either contained no or too few specimens to estimate the size–weight scaling or its confidence interval, or were not significant in the Kendall–Theil robust line fitting, and were thus not included in the analysis. While neither *G. ruber* (white) nor *G. elongatus* show signs of a non-constant size–weight scaling over time (compare Fig. 8), a constant mean calcification in the synthetic taxon (dashed blue line) can only be assumed when disregarding two samples marked with the green arrows. The values generally appear too high from late winter to early summer. Grey boxes with letters at the bottom indicate months, the vertical, dashed, grey line marks the end of 2002.

**Table 8.** Results of the most informative generalized linear Model (4) for the shell size of *Globigerinoides* species from trap L1/K276 in the North Atlantic. The model implies that shell size is not influenced by any factor monitored.

| | Standard error | $t$-value | $p$-value |
|---|---|---|---|
| Intercept | 54.5645 | 2.626 | .009 |
| SST | 2.255 | 1.224 | .221 |
| Chl. a | 79.557 | 1.215 | .225 |
| Biospecies | 88.722 | 1.726 | .085 |
| Shell flux | 0.800 | 1.204 | .229 |
| SST × biospecies | 3.736 | −1.610 | .108 |
| Chl. a × biospecies | 125.981 | 0.413 | .679 |
| Shell flux × biospecies | 1.293 | 0.963 | .336 |

this study is not the best descriptor of optimum growth conditions and relative abundance in relationship to all planktonic Foraminifera in the analysed samples, as used by Hecht (1976), is more appropriate. To this end, we carried out the same tests but used relative abundance data from Storz et al. (2009). Since these were not available for *G. ruber* (white) and *G. elongatus* separately, we only carried out an analy-

sis according to Model (2) for both *Globigerinoides* species. The results are virtually similar to those when using absolute abundance: no significant relationship between relative abundance and size could be found. The same applies even when the analyses of calcification intensity are repeated with relative abundance instead of flux (Suppl. 2, Tables S3–S6). Thus, we conclude that across the range of environmental conditions represented in our study, shell size does not seem to reflect optimum growth conditions, implying that perhaps even the interpretation of the observed relationships between optimum growth conditions and calcification intensity have to be interpreted with caution. The possibility that calcification is not related to optimum growth conditions or optimum growth conditions are difficult to approximate by abundance could explain the inconsistent reaction of calcification to this parameter observed by our and earlier studies (Table 6).

## 5 Conclusions

The size–weight relationship and calcification intensity expressed as area density of individual shells and shell size of *G. ruber* (white), *G. elongatus*, and *G. bulloides* have been analysed in a series of 18 samples from a sediment trap in the Azores Current representing shell flux between spring 2002 to spring 2003. The site represents a range of conditions with respect to temperature, productivity and optimum growth, whilst showing only a small range of variation in carbonate saturation. In this way, it allows us to investigate the effect of parameters other than carbonate saturation on the calcification process in planktonic Foraminifera.

Our data imply that the size–weight scaling in planktonic Foraminifera varies between species, but appears stable across a range of environmental conditions within species. Furthermore, the size–weight scaling is not correlated with calcification intensity, implying that changes in calcification intensity expressed as average area density value for multiple shells in a sample can be interpreted in terms of the effect of potential controlling environmental parameters. We could further show that the previously used method of mean area density agrees with data obtained from individual area density calculations, but that the earlier method underestimates MAD uncertainty. The less labour intensive MAD approach is thus suitable for studies of calcification intensity, but it requires an explicit estimate of uncertainty obtained by direct replication.

An analysis of the calcification intensity with a variety of generalized linear models indicates that the calcification intensity of foraminiferal shells appears to be influenced by a combination of multiple environmental factors, and that the reactions to these parameters are species specific. While in both *Globigerinoides* species calcification seems to respond to temperature and productivity, the calcification of *G. bulloides* recovered from the same samples appear to be negatively correlated with the abundance of the species. Moreover, the calcification intensity of *G. ruber* (white) and *G. elongatus* generally differs and also reacts differently to changes in the potential controlling parameters. Thus, although the size–weight scaling is similar for both species, their differential reaction implies that data based on a pooled analyses, as it has been often done in the past, could reflect changing proportions of those species irrespective of changes in the postulated environmental parameters.

Analyses of shell size variation among the three species indicates that in our data shell size is not related to optimum growth, contradicting current concepts and/or questioning the use of abundance (absolute and relative) as an estimator of optimum growth conditions. These observation could explain the inconsistent relationship between abundance and calcification observed both in this study and in previous investigations, indicating that optimum growth conditions are either difficult to approximate by abundance or not affecting calcification.

*Acknowledgements.* We want to thank Joanna Waniek, who made the samples used in this study available, André Wizemann, who helped in separating *G. ruber* (white) and *G. elongatus*, and Hartmut Schulz for supervision of JGK and fruitful discussions. The principal author received funding from the Ministerium für Wissenschaft, Forschung und Kunst Baden–Württemberg (LGFG stipend) and the Cushman Foundation for Foraminiferal Research (J. M. Resig Fellowship).

## References

Akaike, H.: A new look at the statistical model identification, IEEE T. Automat. Contr., 19, 716–723, doi:10.1109/TAC.1974.1100705, 1974.

Aldridge, D., Beer, Ch. J., and Purdie, D. A.: Calcfication in the planktonic Foraminifera *Globigerina bulloides* linked to phosphate concentrations in surface waters of the North Atlantic Ocean, Biogeosciences, 9, 1725–1739, doi:10.5194/bg-9-1725-2012, 2012.

Aurahs, R., Treis, Y., Darling, K., and Kučera, M.: A revised taxonomic and phylogenetic concept for the planktonic foraminifer species *Globigerinoides ruber* based on molecular and morphometric evidence, Mar. Micropaleontol., 79, 1–14, doi:10.1016/j.marmicro.2010.12.001, 2011.

Barker, S. and Elderfield, H.: Foraminiferal calcification response to glacial–interglacial changes in atmospheric $CO_2$, Science, 297, 833–836, doi:10.1126/science.1072815, 2002.

Bé, A. W. H.: An ecological, zoogeographic and taxonomic review of recent planktonic Foraminifera, in: Oceanic Micropalaeontology, edited by Ramsay, A. T. S., vol. 1, chap. 1, pp. 1–100, Academic Press, London, New York, San Francisco, 1977.

Bé, A. W. H. and Lott, L.: Shell growth and structure of planktonic Foraminifera, Science, 145, 823–824, doi:10.1126/science.145.3634.823, 1964.

Beer, Ch. J., Schiebel, R., and Wilson, P. A.: Technical note: On methodologies for determining the size-normalised weight of planktic Foraminifera, Biogeosciences, 7, 2193–2198, doi:10.5194/bg-7-2193-2010, 2010a.

Beer, Ch. J., Schiebel, R., and Wilson, P. A.: Testing planktic foraminiferal shell weight as a surface water $\left[CO_3^{2-}\right]$ proxy using plankton net samples, Geology, 38, 103–106, doi:10.1130/G30150.1, 2010b.

Benjamini, Y. and Hochberg, Y.: Controlling the false discovery rate: A practical and powerful approach to multiple testing, J. R. Stat. Soc. B Met., 57, 289–300, doi:10.2307/2346101, 1995.

Bijma, J., Spero, H. J., and Lea, D. W.: Reassessing foraminiferal stable isotope geochemistry: Impact of the oceanic carbonate system (experimental results), in: Use of Proxies in Paleoceanography: Examples from the South Atlantic, edited by Fischer, G. and Wefer, G., pp. 489–512, Springer-Verlag, Berlin, Heidelberg, 1999.

Broecker, W. and Clark, E.: An evaluation of Lohmann's Foraminifera weight dissolution index, Paleoceanography, 16, 531–534, doi:10.1029/2000PA000600, 2001a.

Broecker, W. S. and Clark, E.: Reevaluation of the $CaCO_3$ size index paleocarbonate ion proxy, Paleoceanography, 16, 669–671, doi:10.1029/2001PA000660, 2001b.

Brummer, G. J. A. and Kroon, D.: Planktonic Foraminifers as Tracers of Ocean-Climate History: Ontogeny, Relationships and Preservation of Modern Species and Stable Isotopes, Phenotypes and Assemblage Distribution in Different Water Masses, Free University Press, Amsterdam, 1988.

Caron, D. A., Bé, A. W. H., and Anderson, O. R.: Effects of variations in light intensity on life processes of the planktonic foraminifer *Globigerinoides sacculifer* in laboratory culture, J. Mar. Biol. Assoc. UK, 62, 435–451, doi:10.1017/S0025315400057374, 1982.

Darling, K. F. and Wade, Ch. M.: The genetic diversity of planktic Foraminifera and the global distribution of ribosomal RNA genotypes, Mar. Micropaleontol., 67, 216–238, doi:10.1016/j.marmicro.2008.01.009, 2008.

Davis, C. V., Badger, M. P. S., Bown, P. R., and Schmidt, D. N.: The response of calcifying plankton to climate change in the Pliocene, Biogeosciences, 10, 6131–6139, doi:10.5194/bg-10-6131-2013, 2013.

Davison, A. C. and Hinkley, D. V.: Bootstrap Methods and their Applications, vol. 1 of *Cambridge Series in Statistical and Probabilistic Mathematics*, Cambridge University Press, Cambridge, 1997.

de Moel, H., Ganssen, G. M., Peeters, F. J. C., Jung, S. J. A., Kroon, D., Brummer, G.-J. A., and Zeebe, R. E.: Planktic foraminiferal shell thinning in the Arabian Sea due to anthropogenic ocean acidification?, Biogeosciences, 6, 1917–1925, doi:10.5194/bg-6-1917-2009, 2009.

de Villiers, S.: Optimum growth conditions as opposed to calcite saturation as a control on the calcification rate and shell-weight of marine Foraminifera, Mar. Biol., 144, 45–49, doi:10.1007/s00227-003-1183-8, 2004.

Dixon, Ph. M.: Bootstrap resampling, in: Encyclopedia of Environmetrics, edited by El-Shaarawi, A. H. and Piegorsch, W. W., vol. 1, pp. 212–220, John Wiley & Sons, Ltd., Chichester, 2002.

Dormann, C. F., Elith, J., Sven, B., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G., Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E., Reineking, B., Schröder, B., Skidmore, A. K., Zurell, D., and Lautenbach, S.: Collinearity: A review of methods to deal with it and a simulation study evaluating their performance, Ecography, 36, 27–46, doi:10.1111/j.1600-0587.2012.07348.x, 2013.

Erez, J. and Honjo, S.: Comparison of isotopic composition of planktonic Foraminifera in plankton tows, sediment traps and sediments, Palaeogeogr. Palaeocl. Palaeoecol., 33, 129–156, doi:10.1016/0031-0182(81)90035-3, 1981.

Fligner, M. A. and Killeen, T. J.: Distribution-free two-sample tests for scale, J. Am. Stat. Assoc., 71, 210–213, doi:10.1080/01621459.1976.10481517, http://www.jstor.org/stable/2285771, 1976.

Garcia, H. E., Locarnini, R. A., Boyer, T. P., Antonov, J. I., Baranova, O. K., Zweng, M. M., Reagan, J. R., and Johnson, D. R.: World Ocean Atlas 2013, volume 4: Dissolved inorganic nutrients (phosphate, nitrate, silicate), in: NOAA Atlas NESDIS, edited by Levitus, S. and Mishonov, A., vol. 76, pp. 1–25, National Oceanic and Atmospheric Administration, Silver Spring, http://data.nodc.noaa.gov/woa/WOA13/DOC/woa13_vol4.pdf, 2013.

Gonzalez-Mora, B., Sierro, F. J., and Flores, J. A.: Controls of shell calcification in planktonic foraminifers, Quaternary Sci. Rev., 27, 956–961, doi:10.1016/j.quascirev.2008.01.008, 2008.

Goyet, C., Healy, R., Ryan, J., and Kozyr, A.: Global Distribution of Total Inorganic Carbon and Total Alkalinity below the Deepest Winter Mixed Layer Depths, Tech. Rep. 4995, U.S. Department of Energy, http://odv.awi.de/en/data/ocean/global_alkanity_tco2/, 2000.

Hecht, A. D.: An ecologic model for test size variation in recent planktonic Foraminifera: Applications to the fossil record, J. Foramin. Res., 6, 295–311, doi:10.2113/gsjfr.6.4.295, 1976.

Helsel, D. R. and Hirsch, R. M.: Statistical methods in water resources, in: Hydrologic Analysis and Interpretation, vol. 4 of *Techniques of Water-Resources Investigations Reports*, chap. 3, pp. 1–510, United States Geological Survey, 2002.

Ingleby, B. and Huddleston, M.: Quality control of ocean temperature and salinity profiles — historical and real-time data, J. Marine Syst., 65, 158–175, doi:10.1016/j.jmarsys.2005.11.019, 2007.

Kendall, M. G.: A new measurement of rank correlation, Biometrika, 30, 81–93, doi:10.1093/biomet/30.1-2.81, 1938.

Klein, B. and Siedler, G.: On the origin of the Azores Current, J. Geophys. Res.–Oceans, 94, 6159–6168, doi:10.1029/JC094iC05p06159, 1989.

Kremling, K., Lentz, U., Zeitschel, B., Schulz-Bull, D. E., and Duinker, J. C.: New type of time-series sediment trap for the reliable collection of inorganic and organic trace chemical substances, Rev. Sci. Instrum., 67, 4360–4363, doi:10.1063/1.1147582, 1996.

Kruskal, W. H. and Wallis, W. A.: Use of ranks in one-criterion variance analysis, J. Am. Stat. Assoc., 47, 583–621, doi:10.1080/01621459.1952.10483441, 1952.

Kučera, M.: Planktonic Foraminifera as tracers of past oceanic environments, in: Proxies in Late Cenozoic Paleoceanography, edited by Hillaire-Marcel, C., de Vernal, A., and Chamley, H., vol. 1 of *Developments in Marine Geology*, chap. 6, pp. 213–262, Elsevier, Amsterdam, doi:10.1016/S1572-5480(07)01011-1, 2007.

Lewis, E., Wallace, D., and Allison, L. J.: Program Developed for $CO_2$ System Calculations, Tech. Rep. 4735, Carbon Dioxide Information Analysis Center, Oak Ridge, http://cdiac.ornl.gov/oceans/co2rprt.html, 1998.

Locarnini, R. A., Mishonov, A. V., Antonov, J. I., Boyer, T. P., Garcia, H. E., Baranova, O. K., Zweng, M. M., Paver, C. R., Reagan, J. R., Johnson, D. R., Hamilton, M., and Seidov, D.: World Ocean Atlas 2013, volume 1: Temperature, in: NOAA Atlas NESDIS, edited by Levitus, S. and Mishonov, A., vol. 73, pp. 1–40, National Oceanic and Atmospheric Administration, Silver Spring, http://data.nodc.noaa.gov/woa/WOA13/DOC/woa13_vol1.pdf, 2013.

Lohmann, G. P.: A model for variation in the chemistry of planktonic Foraminifera due to secondary calcification selective dissolution, Paleoceanography, 10, 445–457, doi:10.1029/95PA00059, 1995.

Lombard, F., da Rocha, R. E., Bijma, J., and Gattuso, J.-P.: Effect of carbonate ion concentration and irradiance on calcfication in planktonic Foraminifera, Biogeosciences, 7, 247–255, doi:10.5194/bg-7-247-2010, 2010.

Longhurst, A.: Seasonal cycles of pelagic production and consumption, Progr. Oceanogr., 36, 77–167, doi:10.1016/0079-6611(95)00015-1, 1995.

Mann, H. B. and Whitney, D. R.: On a test of whether one of two random variables is stochastically larger than the other, Ann. Math. Stat., 18, 50–60, doi:10.1214/aoms/1177730491, http://www.jstor.org/stable/2236101, 1947.

Manno, C., Morata, N., and Bellerby, R.: Effect of ocean acidification and temperature increase on the planktonic foraminifer *Neogloboquadrina pachyderma* (sinistral), Polar Biol., 35, 1311–1319, doi:10.1007/s00300-012-1174-7, 2012.

Marshall, B. J., Thunell, R. C., Henehan, M. H., Astor, Y., and Wejnert, K. R.: Planktonic foraminiferal area density as a proxy for carbonate ion concentration: A calibration study using the Cariaco Basin Ocean Time Series, Paleoceanography, 28, 1–14, doi:10.1002/palo.20034, 2013.

McDonald, J. H.: Handbook of Biological Statistics, Sparky House Publishing, Baltimore, 2nd edn., http://www.biostathandbook.com/, 2009.

Media Cybernetics, Inc.: Image-Pro® Plus Version 6.0 for Windows™ Start-Up Guide, Silver-Spring, http://web.uvic.ca/ail/techniques/IPPStartUp.pdf, 2006.

Moy, A. D., Howard, W. R., Bray, S. G., and Trull, Th. W.: Reduced calcification in modern Southern Ocean planktonic Foraminifera, Nat. Geosci., 2, 276–280, doi:10.1038/NGEO460, 2009.

Naik, S. S., Naidu, P. D., Govil, P., and Godad, S.: Relationship between weights of planktonic foraminifer shell and surface sater $CO_3^{=}$ concentration during the Holocene and last glacial period, Mar. Geol., 275, 278–282, doi:10.1016/j.margeo.2010.05.004, 2010.

Naik, S. S., Godad, S. P., and Naidu, P. D.: Does carbonate ion control planktonic Foraminifera shell calcification in upwelling regions?, Curr. Sci. India, 101, 1370–1375, http://drs.nio.org/drs/handle/2264/3970, 2011.

Naik, S. S., Godad, S. P., Naidu, P. D., and Ramaswamy, V.: A comparison of *Globigerinoides ruber* calcification between upwelling and non-upwelling regions in the Arabian Sea, J. Earth Syst. Sci., 122, 1153–1159, doi:10.1007/s12040-013-0330-y, 2013.

Nelder, J. A. and Wedderburn, R. W. M.: Generalized linear models, J. R. Stat. Soc., Ser. A–G., 135, 370–384, http://www.jstor.org/stable/2344614, 1972.

Numberger, L., Hemleben, Ch., Hoffmann, R., Mackensen, A., Schulz, H., Wunderlich, J.-M., and Kučera, M.: Habitats, abundance patterns and isotopic signals of morphotypes of the planktonic foraminifer *Globigerinoides ruber* (d'Orbigny) in the Eastern Mediterranean Sea since the Marine Isotopic Stage 12, Mar. Micropaleontol., 73, 90–104, doi:10.1016/j.marmicro.2009.07.004, 2009.

Parker, F. L.: Planktonic foraminiferal species in pacific sediments, Micropaleontology, 8, 219–254, doi:10.2307/1484745, 1962.

Prell, W. L., Martin, A., Cullen, J. L., and Trend, M.: The Brown University Foraminiferal Data Base, IGBP PAGES/World Data Center-A for Paleoclimatology Data Contribution Series 1999-027, NOAA/NGDC Paleoclimatology Program, Boulder, http://www.ncdc.noaa.gov/paleo/metadata/noaa-ocean-5908.html, 1999a.

Prell, W. L., Martin, A., Cullen, J. L., and Trend, M.: The Brown University Foraminiferal Data Base (BFD), doi:10.1594/PANGAEA.96900, 1999b.

R Development Core Team: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, http://www.R-project.org/, 2014.

Robbins, L. L.: Environmental significance of morphologic variability in open-ocean versus ocean-margin assemblages of *Orbulina universa*, J. Foramin. Res., 18, 326–333, doi:10.2113/gsjfr.18.4.326, 1988.

Schiebel, R., Waniek, J., Zeltner, A., and Alves, M.: Impact of the Azores Front on the distribution of planktic foraminifers, shelled gastropods, and coccolithophorids, Deep-Sea Res. Pt. II, 49, 4035–4050, doi:10.1016/S0967-0645(02)00141-8, 2002.

Schlitzer, R.: Ocean Data View, Alfred-Wegener-Institut, http://odv.awi.de, 2014.

Schmidt, D. N., Renaud, S., and Bollmann, J.: Response of planktic foraminiferal size to late Quaternary climate change, Paleoceanography, 18, Article 1039, doi:10.1029/2002PA000831, 2003.

Schmidt, D. N., Renaud, S., Bollmann, J., Schiebel, R., and Thierstein, H. R.: Size distribution of Holocene planktic foraminifer assemblages: Biogeography, ecology and adaptation, Mar. Micropaleontol., 50, 319–338, doi:10.1016/S0377-8398(03)00098-7, 2004.

Sen, P. K.: Estimates of the regression coefficient based on Kendall's tau, J. Am. Stat. Assoc., 63, 1379–1389, http://www.jstor.org/stable/2285891, 1968.

Shapiro, S. S. and Wilk, M. B.: An analysis of variance test for normality (complete samples), Biometrika, 52, 591–611, doi:10.1093/biomet/52.3-4.591, 1965.

Spero, H. J.: Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*, Mar. Biol., 99, 9–20, doi:10.1007/BF00644972, 1988.

Spero, H. J., Bijma, J., Lea, D. W., and Bemis, B. E.: Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes, Nature, 390, 497–500, doi:10.1038/37333, 1997.

Steinke, S., Chiu, H.-Y., Yu, P.-S., Shen, C.-C., Löwemark, L., Mii, H.-S., and Chen, M.-T.: Mg/Ca ratios of two *Globigerinoides ruber* (white) morphotypes: Implications for reconstructing past tropical/subtropical surface water conditions, Geochem. Geophy. Geosy., 6, Q11 005, doi:10.1029/2005GC000926, 2005.

Storz, D.: Die Saisonalität planktischer Foraminiferen im Bereich einer Sinkstoffallenstation in subtropischen östlichen Nordat-

lantik zwischen Februar 2002 bis April 2004, Diploma thesis, Eberhard–Karls Universität Tübingen, Tübingen, 2006.

Storz, D., Schulz, H., Waniek, J. J., Schulz-Bull, D. E., and Kučera, M.: Seasonal and interannual variability of the planktic foraminiferal flux in the vicinity of the Azores Current, Deep-Sea Res. Pt. I, 56, 107–124, doi:10.1016/j.dsr.2008.08.009, 2009.

Tabor, J.: Investigating the investigative task: Testing for skewness—an investigation of different test statistics and their power to detect skewness, J. Stat. Educ., 18, 1–13, www.amstat.org/publications/jse/v18n2/tabor.pdf, 2010.

Theil, H.: A rank-invariant method of linear and polynomial regression analysis, III, P. K. Akad. Wet.–Amsterd., 53, 1397–1412, 1950.

Wang, L.: Isotopic signals in two morphotypes of *Globigerinoides ruber* (white) from the South China Sea: Implications for monsoon climate change during the last glacial cycle, Palaeogeogr. Palaeocl. Palaeoecol., 161, 381–394, doi:10.1016/S0031-0182(00)00094-8, 2000.

Waniek, J. J., Schulz-Bull, D. E., Blanz, Th., Prien, R. D., Oschlies, A., and Müller, Th. J.: Interannual variability of deep water particle flux in relation to production and lateral sources in the northeast Atlantic, Deep-Sea Res. Pt. I, 52, 33–50, doi:10.1016/j.dsr.2004.08.008, 2005.

Weinkauf, M. F. G., Moller, T., Koch, M. C., and Kučera, M.: Calcification intensity in planktonic Foraminifera reflects ambient conditions irrespective of environmental stress, Biogeosciences, 10, 6639–6655, doi:10.5194/bg-10-6639-2013, 2013.

Yoder, J. and Kennelly, M.: Live Access to US JGOFS SMP Data: Global SeaWiFS chlorophyll, Tech. rep., U.S. JGOFS, http://usjgofs.whoi.edu/las?dset=Ocean+Color/Global+SeaWiFS+chlorophyll+1997-2004, 2005.

Yohai, V. J., Stahel, W. A., and Zamar, R. H.: A procedure for robust estimation and inference in linear regression, in: Directions in Robust Statistics and Diagnostics, Part II, edited by Stahel, W. A. and Weisberg, S., vol. 34 of *The IMA Volumes in Mathematics and its Applications*, pp. 365–374, Springer-Verlag, New York, doi:10.1007/978-1-4612-4444-8_20, 1991.

Zweng, M. M., Reagan, J. R., Antonov, J. I., Locarnini, R. A., Mishonov, A. V., Boyer, T. P., Garcia, H. E., Baranova, O. K., Johnson, D. R., Seidov, D., and Biddle, M. M.: World Ocean Atlas 2013, volume 2: Salinity, in: NOAA Atlas NESDIS, edited by Levitus, S. and Mishonov, A., vol. 74, pp. 1–39, National Oceanic and Atmospheric Administration, Silver Spring, http://data.nodc.noaa.gov/woa/WOA13/DOC/woa13_vol2.pdf, 2013.

# Chapter 6

**Manuscript 3**: Weinkauf, M. F. G., Moller, T., Koch, M. C., and Kučera, M. (2014) Disruptive selection and bet-hedging in planktonic Foraminifera: Shell morphology as predictor of extinctions, *Frontiers in Ecology and Evolution* 2: Article 64, doi:10.3389/fevo.2014.00064

## Abstract

Extinction is a remarkably difficult phenomenon to study under natural conditions. This is because the outcome of stress exposure and associated fitness reduction is not known until the extinction occurs and it remains unclear whether there is any phenotypic reaction of the exposed population that can be used to predict its fate. Here we take advantage of the fossil record, where the ecological outcome of stress exposure is known. Specifically, we analyze shell morphology of planktonic Foraminifera in sediment samples from the Mediterranean, during an interval preceding local extinctions. In two species representing different plankton habitats, we observe shifts in trait state and decrease in variance in association with non-terminal stress, indicating stabilizing selection. At terminal stress levels, immediately before extinction, we observe increased growth asymmetry and trait variance, indicating disruptive selection and bet-hedging. The pre-extinction populations of both species show a combination of trait states and trait variance distinct from all populations exposed to non-terminal levels of stress. This finding indicates that the phenotypic history of a population may allow the detection of threshold levels of stress, likely to lead to extinction. It is thus an alternative to population dynamics in studying and monitoring natural population ecology.

frontiers in
ECOLOGY AND EVOLUTION

ORIGINAL RESEARCH ARTICLE
published: 14 October 2014
doi: 10.3389/fevo.2014.00064

# Disruptive selection and bet-hedging in planktonic Foraminifera: shell morphology as predictor of extinctions

***Manuel F. G. Weinkauf[1,2]*, Tobias Moller[1], Mirjam C. Koch[1] and Michal Kučera[2]***

[1] *Micropalaeontology, Department of Geosciences, Eberhard–Karls University, Tübingen, Germany*
[2] *Center for Marine Environmental Sciences, Micropalaeontology-Palaeoceanography, University Bremen, Bremen, Germany*

***Edited by:***
*G. Lynn Wingard, U.S. Geological Survey, USA*

***Reviewed by:***
*Pasquale Raia, Università di Napoli Federico II, Italy*
*Teresa Vegas-Vilarrúbia, University of Barcelona, Spain*

****Correspondence:***
*Manuel F. G. Weinkauf, Center for Marine Environmental Sciences, University Bremen, Leobener Str., Bremen 28359, Germany*
*e-mail: mweinkauf@marum.de*

Extinction is a remarkably difficult phenomenon to study under natural conditions. This is because the outcome of stress exposure and associated fitness reduction is not known until the extinction occurs and it remains unclear whether there is any phenotypic reaction of the exposed population that can be used to predict its fate. Here we take advantage of the fossil record, where the ecological outcome of stress exposure is known. Specifically, we analyze shell morphology of planktonic Foraminifera in sediment samples from the Mediterranean, during an interval preceding local extinctions. In two species representing different plankton habitats, we observe shifts in trait state and decrease in variance in association with non-terminal stress, indicating stabilizing selection. At terminal stress levels, immediately before extinction, we observe increased growth asymmetry and trait variance, indicating disruptive selection and bet-hedging. The pre-extinction populations of both species show a combination of trait states and trait variance distinct from all populations exposed to non-terminal levels of stress. This finding indicates that the phenotypic history of a population may allow the detection of threshold levels of stress, likely to lead to extinction. It is thus an alternative to population dynamics in studying and monitoring natural population ecology.

**Keywords: environmental stress, fluctuating asymmetry, growth symmetry, morphology, selection, planktonic Foraminifera, Mediterranean**

## INTRODUCTION

Being able to predict impending local extinctions in recent ecosystems could significantly enhance current techniques of biomonitoring. However, although recently large advances in the field of population dynamics channeling into extinction prediction has been made (Drake and Griffen, 2010) this application still suffers from the naturally large variability in population sizes (Ludwig, 1999). Indicators other than population dynamics may be more suitable as predictors for rising stress levels and local extinctions.

It has long been hypothesized that certain aspects of morphology, such as the continuity of growth regularity (fluctuating asymmetry, FA), are influenced by environmental stress (Furlow et al., 1997; Leung et al., 2000). Environmental stress is in this context defined as the degree of deviation of all environmental factors (biotic and abiotic) from the optimum requirements of a species. This hypothesis has been supported by studies where developmental instability was shown to be correlated with fitness (Lens et al., 2002; Hendrickx et al., 2003). In this context a decrease in variability is commonly attributed to stabilizing selection, often associated with reduced environmental variability (Van Valen, 1965), though it can also occur under fluctuating selection in an unstable, continuously changing environment (Pélabon et al., 2010). Increasing variability, on the other hand, is associated with disruptive selection (Bull, 1987), and can thus be the long-term result of bet-hedging of specimens that produce offspring with a higher inter-individual variability, thus increasing the mean fitness of the population (Slatkin, 1974; Philippi and Seger, 1989; Grafen, 1999). Specifically, two different modes of bet-hedging can be distinguished (Einum and Fleming, 2004): (1) conservative bet-hedging, where the populations shows a directional developmental trend toward a state that would reduce fitness under optimal conditions but increases fitness under the prevailing parameters (Einum and Fleming, 1999), and (2) diversified bet-hedging where the population increases its variance so that the chances of at least some individuals to survive are maximized (Philippi and Seger, 1989).

Under natural conditions, it is difficult to assess at what stress levels stabilization yields to disruption. This is partly because it is difficult to identify suitable natural experiments, but most importantly because it is difficult to predict the outcome of stress exposure and thus stress severity on ecological time scales (Moritz and Agudo, 2013). The latter constraint does not apply to the fossil record, where the outcome of stress exposure can be directly observed. Unfortunately, in many cases the fossil record does not have the resolution and richness needed to assess morphological change acting on a spatially well constrained population. Additionally, when working with fossil material the fossilization potential of different biomaterials under varying depositional conditions can bias the preservation of the fossil record that can be used for such a study. In this respect, marine microplankton, such as planktonic Foraminifera, offer the best model system. Foraminifera possess intricate geometrical shells consisting of sequentially accreted chambers, thus reflecting aspects of individual growth, despite their unicellular grade of organization. The

calcite shells of planktonic Foraminifera are preserved in large quantities in marine sediments deposited above the carbonate compensation depth, leaving a highly representative record of population changes through time (e.g., Kučera, 2007).

Studies of recent planktonic Foraminifera have shown abundant evidence for changes in shell traits along environmental gradients (Malmgren and Kennett, 1976), suggesting that individual growth characteristics in these organisms react to changes in environmental parameters. The geometry of Foraminifera shells is given by cytoskeleton assembly during the calcification of each new chamber (Bé et al., 1979). Under extreme stress, e.g., poisoning by heavy metals, the ability of Foraminifera to constrain the shape of their shells is limited, leading to aberrant morphologies (e.g., Caron et al., 1987; Alve, 1991). These effects are most likely associated with direct cytotoxicity, interfering with cytoskeleton assembly. Although no experimental studies exist that assess the influence of natural levels of stress on the geometry of Foraminifera shells, it is likely that their growth regularity may be affected. In multicellular organisms, it is known that exposure to environmental stress can decrease the ability of chaperones to facilitate the conformation of structurally relevant enzymes (Debat et al., 2006). This effect can lead to morphological deviations during the growth of an organism, and can thus serve as a proxy for the occurrence of such stress during the lifetime of the organism (Hendrickx et al., 2003; Beasley et al., 2013).

In this study we present a morphometric analysis of two species of planktonic Foraminifera during the onset of the deposition of Sapropel S5 in the Eastern Mediterranean Sea (**Figure 1**) (Cane et al., 2002). The oxygenation of the deeper water column in this area is facilitated by thermal sinking of saline (39‰) surface waters (Rohling et al., 2009). About 124 kyrs ago, a strengthened monsoon over Africa significantly reduced the surface water salinities in the Eastern Mediterranean due to a distinct rise in freshwater inflow via the Nile (Williams et al., 1978). This process left a distinct signature in the form of significantly lighter stable oxygen isotope values of the upper water column. The excess freshwater influx led to a reduction of surface water salinity from ca 39 psu to ca 35 psu (Van Der Meer et al., 2007), preventing deep water formation. The resulting stagnation of the vertical circulation led to deep water anoxia and the deposition of organic rich sapropel layers (Rossignol-Strick et al., 1982; Rohling, 1994). Because of common forcing by increasing summer insolation, contemporaneously to the onset of the sapropel deposition, local sea surface temperature (SST) rose by approximately 3°C (Marino et al., 2007). In response to these events, local extinctions of several species of planktonic Foraminifera occurred throughout the entire Eastern Mediterranean (**Figure 1**) (Cane et al., 2002). It must be made clear that those local extinctions do not belong to any kind of absolute extinction as described by Delord (2007), but are rather regional disappearances of the species within the Eastern Mediterranean. Many if not all of those species reinvaded the Eastern Mediterranean at a later point when environmental conditions switched back to a previous state, and all those species still exist until the present day at least in other regions of the world. Since those local extinctions appeared on a regional scale, and are therefore not merely the result of migration patterns, however, marine sediments with fossils of Foraminifera from this period provide a direct record of a natural stress experiment that took place over ecological time scales, which could not be simulated during laboratory experiments. This advantage comes at the price, however, that the natural experiment can only be observed at limited time resolution and that the sampling is affected by temporal averaging. In addition, our ability to identify the stressors responsible for the reaction of the ecosystem is limited.

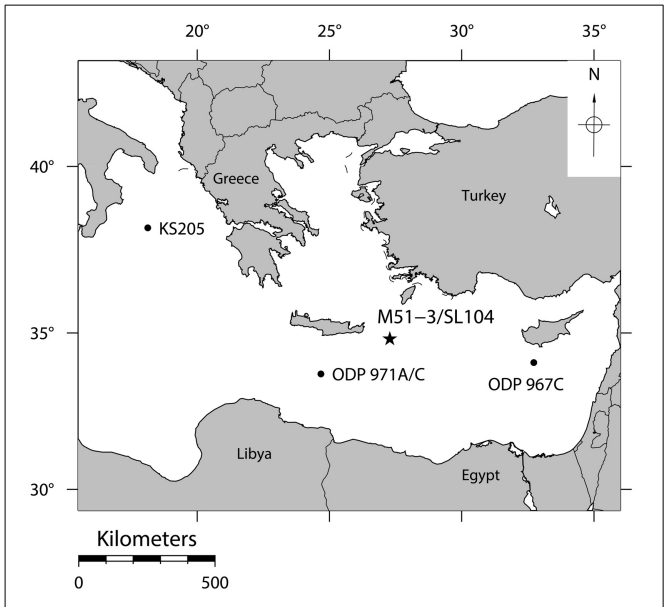



**FIGURE 1 | The position of core M51-3/SL104 in the Pliny Trench about 100 km east-south-east of Crete where the reaction of planktonic Foraminifera to stress has been studied.** Positions of other cores, where the same local extinctions of the species investigated here have been observed (Cane et al., 2002), are indicated by black dots.

In an earlier study Weinkauf et al. (2013) have shown, that in the same environmental setting, the terminal stress level (i.e., a severity of stress that leads to local disappearance of the species) did not influence the ability of the Foraminifera to calcify their shells on a cellular level, but the events associated with the onset of the sapropel deposition had an effect on the calcification intensity of the Foraminifera. Since shell calcification in planktonic Foraminifera is strictly controlled by the living cell (e.g., Bé et al., 1979; Spero et al., 1991) this is likely to reflect the influence of a physiological process associated with the environmental change, rather than just a passive environmental effect. It is thus reasonable to assume that the shell morphology might have been influenced as well. Our hypothesis is, therefore, that morphological changes associated with different levels of stress occurring around the onset of Sapropel S5 should be detectable in the fossil record of planktonic Foraminifera. If this is the case, the state of the populations recorded shortly prior to extinction should reflect the phenotypic response of the exposed population to a terminal level of stress, when compared to the unstressed replicates before.

## MATERIALS AND METHODS

### CHOICE OF SAMPLING INTERVAL AND SPECIES

The sampling interval and species (*Orbulina universa* and *Globorotalia scitula*, **Figure 2**) for this study were chosen to cover a

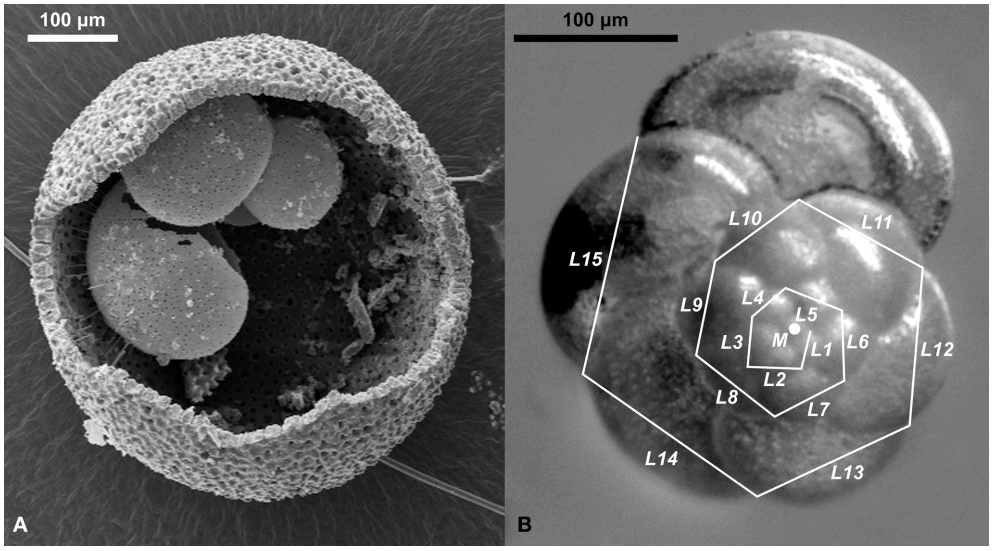



**FIGURE 2 | Depiction of species used in this study. (A)** Scanning electron micrograph of a cracked open shell of *Orbulina universa*, showing the delicate trochospiral juvenile shell preserved within the spherical terminal chamber. **(B)** Measurement of the incremental growth in *Globorotalia scitula*. Straight lines were drawn between consecutive chamber sutures and their length was measured (*L*1–*L*15). The relative change of length between individual line segments was used to calculate the incremental growth *IG* (mean relative length change between consecutive line segments) and an incremental growth variance *IGV* (standard deviation of *IG*) per specimen. The coordinates of the sutures and the spiral center (*M*) were used to describe the deviation of growth from the logarithmic spiral.

wide ecological spectrum and to replicate the observations on the impact of terminal stress leading to local extinction. By including those species in our analysis over an interval that covers several centuries before and after the onset of sapropel deposition we can assess the impact of the presumed salinity shift in the upper water column, as well as the impact of vertical stagnation on the lower water column.

*Orbulina universa* is a shallow-dwelling, symbiont-bearing species with dwelling depths of 20–100 m (Pujol and Vergnaud Grazzini, 1995; Rohling et al., 2004). The species survived the onset of Sapropel S5, but shows two local extinctions within the sapropel (**Figure 3**) (Cane et al., 2002). *Orbulina universa* is known to be associated with cryptic speciation encompassing at least three distinct genetic types (De Vargas et al., 1999). Although only one genetic type was found in the Mediterranean so far (De Vargas et al., 1999), we cannot exclude the possibility, that cryptic speciation influenced the results obtained in our analysis. If this would be relevant for our analysis, however, we should observe a bi- or multimodal distribution of measurement values, with changing mode amplitudes indicating that any observed change in the mean value of morphometric data is the result of changing dominances of sub-populations within the community. A unimodal distribution, on the other hand, indicates a (at least morphologically) homogeneous community that shows a consolidated reaction regardless of their potential cryptic diversity. Consequently, we used Hartigan's dip test (Hartigan and Hartigan, 1985) to test the morphometric data for unimodality.

*Globorotalia scitula* is a deeper dwelling species without symbionts, which shows a local extinction contemporaneous with sapropel onset (**Figure 3**). It was found to be most abundant between 200 and 500 m water depth in the Azores region (Schiebel et al., 2002), which is in good agreement with its reconstructed depth habitat during the deposition of Sapropel S5 (Rohling et al., 2004). The genetic diversity of *G. scitula* has not yet been assessed in detail.

The sampling interval covers the onset of Mediterranean Sapropel S5 at around 126.4 ka, which is visible by both the sediment becoming considerably darker in color and a drop of the $\delta^{18}$O values of shells of *Globigerinoides ruber* (white) from 0.5 ‰ to −1.4 ‰. Specimens of *G. scitula* became locally extinct with the onset of the sapropel, and specimens of that species therefore only cover pre-sapropel conditions in our study, covering a time-span of nearly 1800 years. *Orbulina universa* survived the sapropel onset to become locally extinct later, and thus covers the same time period as *G. scitula* before sapropel onset and an additional 2000 years within the sapropel.

## SAMPLE PROCESSING

The samples were taken from a portion of gravity core M51-3/SL104, taken in the Pliny Trench east-south-east of Crete in the Eastern Mediterranean (**Figure 1**) (Hemleben et al., 2003), covering the onset of Sapropel S5 (Moller et al., 2012). The sediment was deposited ca. 126–121 kyrs ago, and an age model was fitted using a combination of event-stratigraphy (Cane et al., 2002) and layer counting in the laminated part of the sapropel (Moller, 2012). The sampling was performed with a spatial resolution of 3 mm, which corresponds to a temporal resolution of 60–70 yrs in the majority of the section, and about 11 yrs in the topmost 6 cm of the studied interval, covering only the second local extinction of *O. universa*. The samples were washed using tap water and dry sieved, only the fraction $> 150\,\mu$m was used for subsequent analyses.

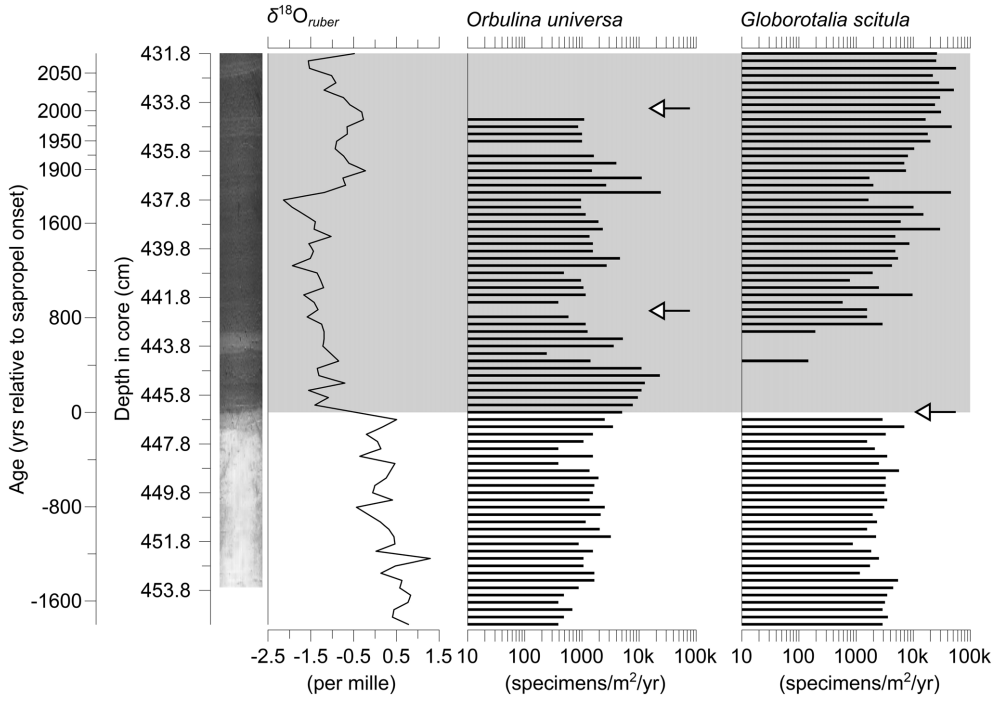



**FIGURE 3 | Accumulation rates of shells of the two species of planktonic Foraminifera in core M51-3/SL104 investigated in this study.** The $\delta^{18}$O values of shells of *Globigerinoides ruber* (white) from the same core serve as a geochemical marker for freshwater injection marking the onset of the sapropel. Local extinctions occurring in the entire Eastern Mediterranean (Cane et al., 2002) are marked with arrows. The gray-shaded area indicates the extent of Sapropel S5, a core-photograph is provided for visualization.

Foraminifera were picked from representative aliquots of the washed residue, split with a binary microsplitter if necessary. *Orbulina universa* was picked from 455.5–434.8 cm (70 samples) and yielded 2025 specimens. Of *G. scitula* 1290 specimens were sampled, ranging from 455.5–447.1 cm (29 samples). The abundance of each species in the analyzed aliquots was determined on the basis of faunal count data assessed from 12.5–100% of the sample volume. Absolute abundances were converted to accumulation rates per square meter per year, using the inferred age model and the cross-sectional area of the samples, assuming constant sample thickness.

## MORPHOMETRIC DATA ACQUISITION

All specimens were oriented in standard taxonomic view, mounted on glass slides using double sided adhesive tape, and photographed under constant magnification with a Leica Z16 stereomicroscope and the Image-Pro® Plus software. All specimens were photographed using transmitted light, specimens of *G. scitula* from selected samples were additionally photographed under reflected light to enable analysis of growth patterns.

*Orbulina universa* is characterized by a trochospiral juvenile shell, that in the terminal life stage is completely overgrown by a spherical terminal chamber, so that adult specimens (which were used in our study exclusively) normally resemble spheres (**Figure 2**). Abnormal morphotypes are known to exist and believed to occur under stress conditions (Caron et al., 1987). Those include "*Orbulina suturalis*," in which part of the juvenile shell is not covered by the terminal chamber, and "*Biorbulina bilobata*," in which the adult shell is composed of two joint hemispheres (**Figure 4**). In *O. universa* the mean diameter and roundness (expressed as ratio between longest and shortest axis) of the shell, and the incidence of the abnormal morphotypes were determined.

*Globorotalia scitula* grows a flat, trochospiral shell (**Figure 2**). In this species the cross-sectional shell area, incidence of antisymmetry (left-coiling), and Kummerforms (specimens in which the last chamber is smaller than the penultimate chamber, **Figure 4**) were determined.

Using reflected light images, the incremental growth of *G. scitula* in selected samples was analyzed by drawing $n$ straight line segments of length $l_i$ between consecutive chamber sutures along the shell outline (**Figure 2**). The relative length relationship between consecutive lines per specimen was then used to calculate the incremental growth $IG$ (mean relative growth, Equation 1) and incremental growth variance $IGV$ (intra-individual standard deviation of relative growth, Equation 2) for each specimen. Only specimens in which at least five consecutive line segments could be measured were used for the analysis. The spiral formed by the $x$–$y$-coordinates of sutures (normalized for a standard spiral-diameter of one) was used to estimate the deviation of the shell from the logarithmic spiral. The $R^2$ value of a ranged major axis regression line (Legendre and Legendre, 2012) through the logarithmically plotted points (log-$R^2$) provided an objective measure of that deviation.

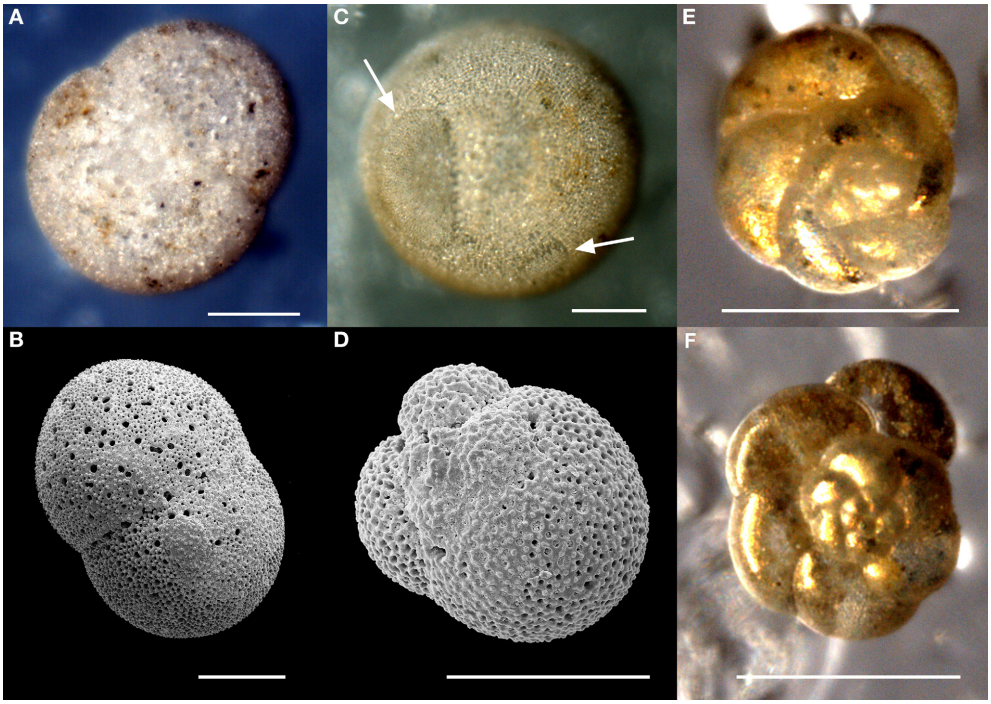



**FIGURE 4 | Depiction of abnormal morphotypes. (A,B)** Specimens of "*Biorbulina bilobata*," an abnormal morphotype of *Orbulina universa* where the adult shell consists of two hemispheres that are joined in the middle, in light microscopic **(A)** and scanning electron microscopic **(B)** view. **(C,D)** Specimens of "*Orbulina suturalis*," an abnormal morphotype of *Orbulina universa* where the juvenile trochospiral shell (compare **Figure 2**) is not completely enclosed by the adult spherical shell, in light microscopic **(C)** and scanning electron microscopic **(D)** view. The protruding juvenile spiral has been marked by arrows in the light microscopy image. **(E,F)** Light microscopy images of Kummerforms of *Globorotalia scitula*, where the last chamber is smaller than the penultimate chamber. While the specimen in **(F)** shows a regular growth pattern (low *IGV*) the specimen in **(E)** shows an increased *IGV* visible by the large variation in the size of individual chambers. Scale bars are 200 μm in length.

$$IG = \frac{\sum_{i=2}^{n} l_i/l_{i-1}}{n-1} \quad (1)$$

$$IGV = \sqrt{\sum_{i=1}^{n} \frac{(l_i/l_{i-1} - IG)^2}{n-2}} \quad (2)$$

The chosen parameters allow us to assess the influence of environmental stress on the morphology of those two foraminifer species on several levels: (*a*) body size appears to reflect environmental stress in planktonic Foraminifera, because it is known to decrease away from the thermal optimum of each species (Schmidt et al., 2004). (*b*) The incidence of abnormal morphotypes (including antisymmetry) is likely to increase under stress indicating disruption of morphogenesis. (*c*) The roundness in *O. universa* and *IG*, *IGV*, and log-$R^2$ in *G. scitula* are an imprint of the FA in those organisms, which is a proxy for environmental stress in multicellular organisms (Leung et al., 2000).

## DATA ANALYSIS

Statistical analyses of the results were performed in R v. 3.0.1 (R Development Core Team, 2013). Confidence intervals of morphological parameters were calculated by bootstrapping using the package "boot" v. 1.3-9 (Davison and Hinkley, 1997), with bootstrapping method choice based on skewness evaluation following Dixon (2002). Skewness (*SK*) was calculated according to recommendations in Tabor (2010) (equation I, table 1), its standard deviation *SDK* for $n$ data values was approximated as $SDK = 2 \times (6/n)^{\frac{1}{2}}$ (Tabachnick and Fidell, 1996). The skewness of the data was considered significant when $SK > SKD$. When the skewness was significant, the basic bootstrap, otherwise the accelerated bootstrap, was used.

For all relative abundances, 95% confidence intervals for multinomial proportions were calculated (Heslop et al., 2011). The normality of data distribution was tested using a Shapiro–Wilk test (Shapiro and Wilk, 1965) wherever necessary to decide between the applicability of parametric or non-parametric tests. Comparisons between the morphological characteristics between two groups (i.e., specimens before and after sapropel onset) were performed by a Mann–Whitney *U* test (Mann and Whitney, 1947).

Morphological growth parameters in *G. scitula* where tested for deviations per sample using Grubb's test for outliers (Grubbs, 1969). To test for the development of increasing (disruptive selection) or decreasing (stabilizing selection) morphological plasticity as result of the environmental stress, we calculated the standard deviation including its 95% confidence interval (Sheskin, 2011) for each of the morphological parameters in each sampling level.

## RESULTS

In both species investigated local disappearances could be observed as expected. *Orbulina universa* shows two such disappearances, both within the sapropel, while *G. scitula* disappeared immediately before sapropel onset (**Figure 3**). In both cases there were no signs of dissolution visible on the foraminiferal shells, with even specimens of fragile, thin-walled species being in a pristine state with transparent shell walls. We further note the occurrence of pteropods throughout the whole sampling interval, which are very susceptible against dissolution due to the aragonitic nature of their shells, and have therefore no reason to assume that the disappearances we observe are the result of diagenetic processes instead of local extinctions.

In *O. universa*, mean shell size showed no obvious difference between pre- and post-sapropel populations ($p = 0.459$) or at local extinctions (**Figure 5**), but the distribution of individual

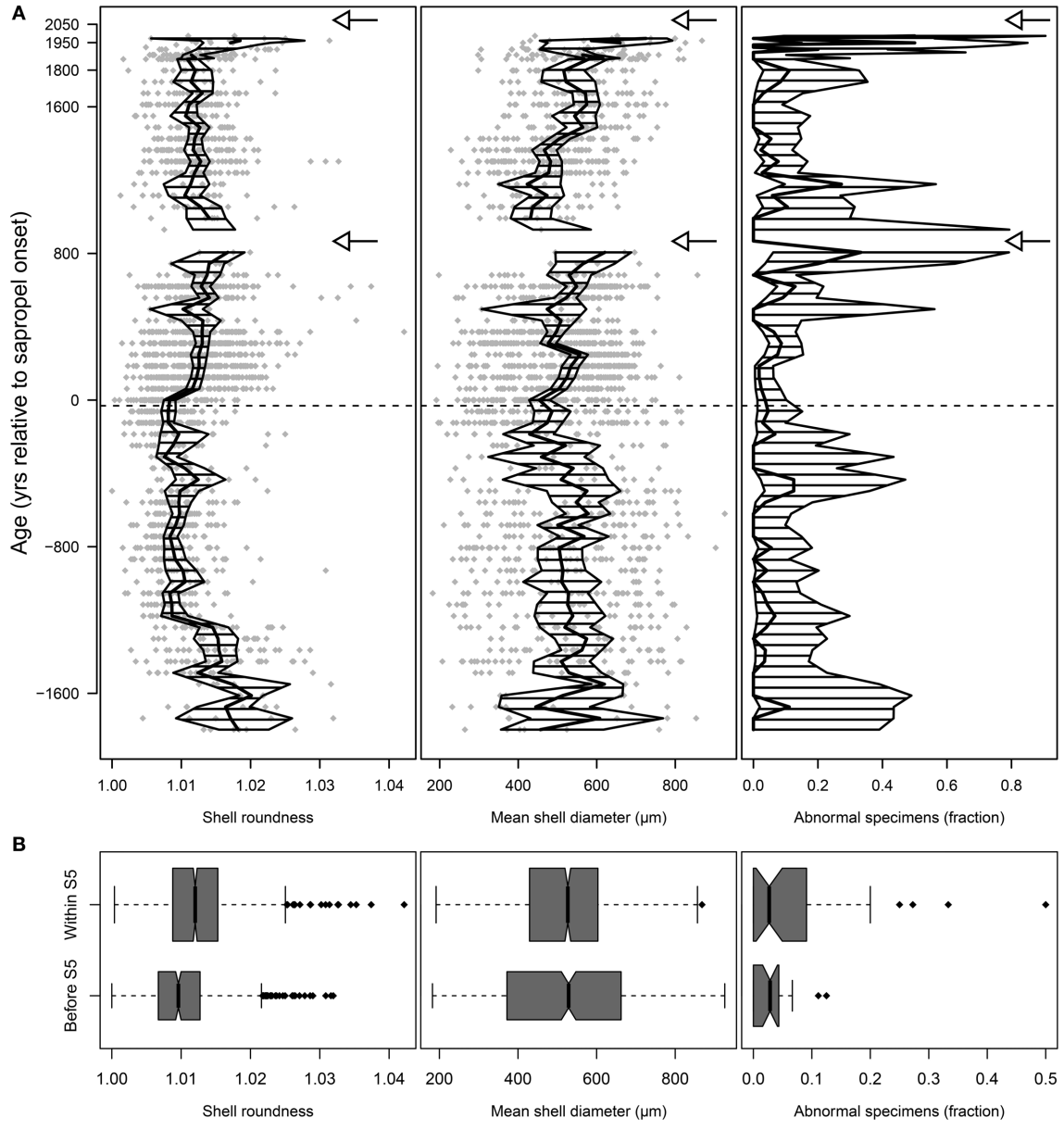



**FIGURE 5 | Morphological analyses of shells of *Orbulina universa* from core M51-3/SL104.** Shell roundness is the ratio between long and short axis of the shell, a value of 1.0 representing a perfect sphere. The incidence of abnormal morphotypes combines counts of specimens of the two ecophenotypes "*Biorbulina bilobata*" and "*Orbulina suturalis*," the arrows mark the local extinctions of the species. **(A)** Stratigraphic plots depicting the raw values for shell roundness and diameter (gray diamonds), the arithmetic mean values (thick black line), and the 95% confidence intervals of the mean (hatched area). The horizontal dashed line marks the onset of Sapropel S5. Note the increasing abundance of abnormal morphotypes before both local extinctions. **(B)** Distribution of values before and within the sapropel. The boxes show the median (thick black line), interquartile range (box), and 1.5 × interquartile range (whiskers); black diamonds mark outliers. Box widths are proportional to the number of within-groups observations. Note the difference in mean shell roundness and average incidence of abnormal morphotypes, but not in mean shell diameter within the sapropel.

values indicates a significant decrease in variance ($p < 0.001$) with the onset of the sapropel (**Figure 5B**, Supplementary Figure 3A). The mean roundness of the terminal chamber changes with sapropel onset (**Figure 5**, Supplementary Figure 3A). The difference between pre- and post-sapropel conditions is significant at $p < 0.001$ but it is not associated with a change in variance ($p = 0.501$).

Within the sapropel, the abundance of abnormal morphotypes of *O. universa* is generally increased in comparison to pre-sapropel conditions ($p = 0.015$, two-proportions $z$-test). Furthermore, the incidence of abnormal morphotypes appears to be highest in samples immediately preceding both local extinctions. Although the associated confidence intervals of the relative abundances of "*O. suturalis*" and "*B. bilobata*" are large (**Figure 5A**), indicating the possibility of a spurious effect, a randomization test, in which randomly selected three-sample groups were compared to each of the three-sample groups before both local extinctions, indicate a significance of the increased incidence of abnormal morphotypes for the second local extinction ($p = 0.011$), but not for the first one ($p = 0.277$).

*Globorotalia scitula* showed no reaction in shell size toward its local extinction. The species also showed no significant reaction in the incidence of left-coiled specimens ($p = 0.138$ for the last two samples before local extinction) or Kummerforms at that time (**Figure 6A**).

Conversely, the values of *IG* and *IGV* were increasing when the species approached its local extinction (**Figure 6B**). Hypothetically it could be assumed, that from some point when

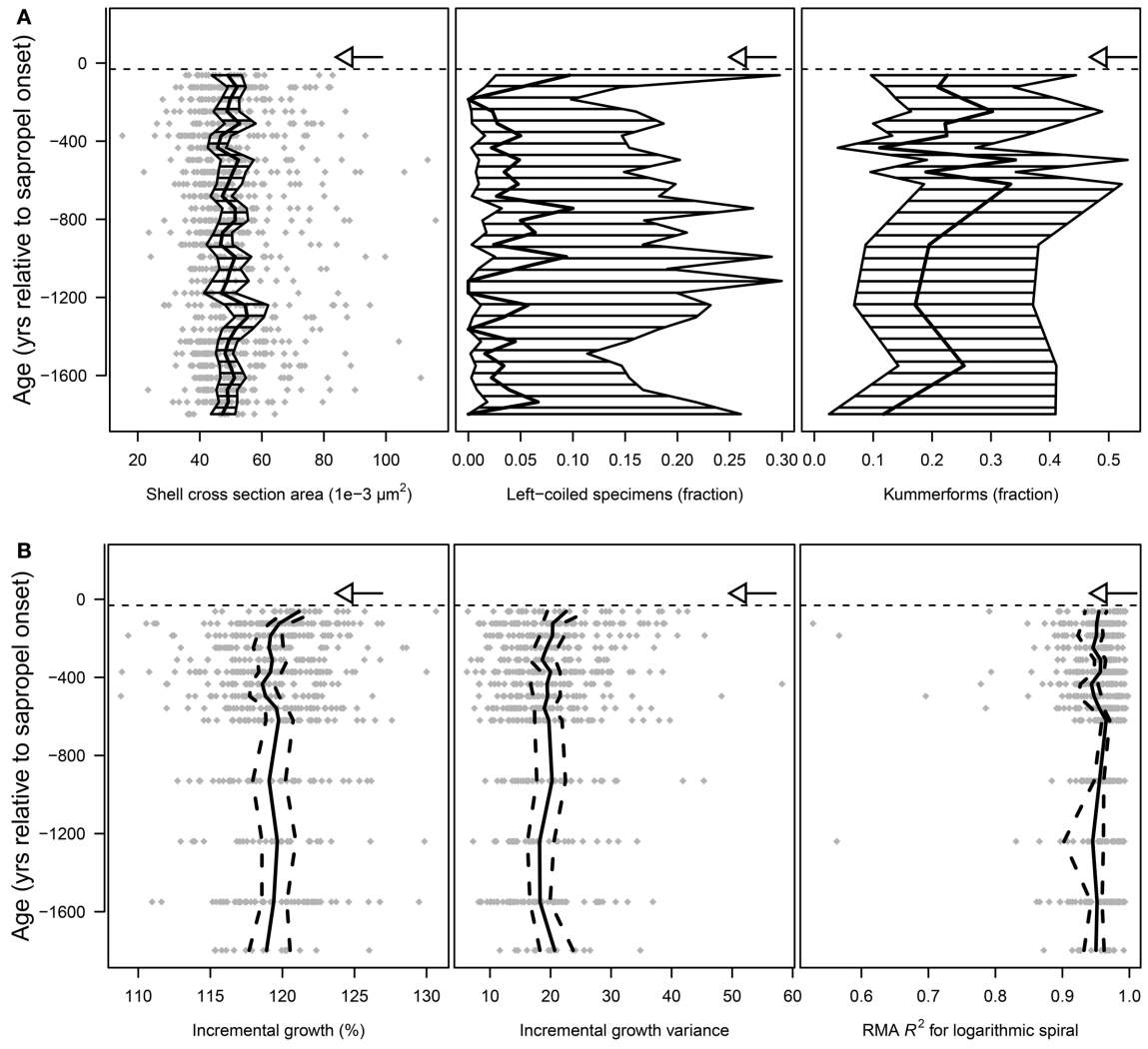



**FIGURE 6 | Morphological analyses of shells of *Globorotalia scitula* from core M51-3/SL104.** Kummerforms are specimens in which the last chamber is smaller than the penultimate chamber. The horizontal dashed lines mark the onset Sapropel S5, the arrows mark the local extinction of the species. **(A)** Arithmetic mean values (thick black line) and 95% confidence intervals of the mean (hatched area), and in the case of shell size the raw values as gray diamonds. **(B)** Raw values (gray diamonds) and mean (solid black line) with 95% confidence interval (dashed black lines) of the incremental growth (*IG*) and its standard deviation (*IGV*) and the deviation of growth from the logarithmic spiral. Both *IG* and *IGV* rise significantly when *G. scitula* approaches its local extinction, whereas the consistency of the logarithmic growth remains the same (compare Supplementary Table 1).

the species approaches its local extinction, a deviation in the growth pattern could be observable, when compared to the background value that prevailed before. To that end, we applied Grubb's test for outliers to check if such a pattern can be recognized in our samples. One-tailed $p$-values for this analysis show that a significant change in *IG* ($p = 0.002$) and *IGV* ($p = 0.024$) only occurs in the very last sample where the species was found, i.e., about 60 years prior to its local extinction (Supplementary Table 1). Disregarding those outliers the rest of the data is normally distributed ($p_{IG} = 0.781$, $p_{IGV} = 0.518$), so that the assumptions to apply Grubb's test are not violated. Furthermore, while the whole community shows a consistent increase in *IG*, the population-wide variance of *IGV* is also increasing when the species approaches local extinction (**Figure 6B**, Supplementary Figure 3B). The growth symmetry, measured by the deviation of the growth spiral from an ideal logarithmic spiral, shows in contrast no reaction when the species approached its local extinction ($p = 0.487$, **Figure 6B**, Supplementary Table 1, data are normally distributed at $p_{R^2} = 0.678$).

## DISCUSSION

### RELIABILITY AND NATURE OF OBSERVED MORPHOLOGICAL PATTERNS

Although not all of the observed morphological variance within Foraminifera can be explained by genetic diversity (André et al., 2013; Mary and Knappertsbusch, 2013) a high genetic variability within Foraminifera was revealed (De Vargas et al., 1999; Aurahs et al., 2011), so that changing morphologies could theoretically be the result of changing dominances of different genotypes. Although until now only one genetic type of *O. universa* is known to occur in the Mediterranean (De Vargas et al., 1999), the trait shifts observed in that species could be the result of a non-constant mixing of different genetic types with different morphologies. However, since we did not find any coherent signal of non-unimodality in the size and roundness measurements (Supplementary Table 2, Supplementary Figures 1, 2), we assume that the analyzed specimens derive from one homogenous community, within which the traits changed with sapropel onset. The results we obtained during our analyses are thus robust with regard to potential genotypic variation and speciation.

We interpret the reduced shell roundness with constant inter-individual variance in *O. universa* (**Figures 5B**, **7A**) as a signal of a permanently increased FA in its population during sapropel times. Conversely, the constant shell size with significantly reduced inter-individual variance during sapropel times (**Figures 5B**, **7A**) indicates stabilizing selection on that trait. The increased incidence of abnormal morphotypes immediately before local extinction (**Figures 5**, **7A**) is distinct from both patterns and is more likely a reflection of excessive conservative bet-hedging.

The increase in mean values of incremental growth and its variance shortly before the local extinction of *G. scitula* (**Figures 6B**, **7B**) signifies an increase in FA. While the inter-individual variance of *IG* was unaffected by the observed local extinction, the inter-individual variance of *IGV* was drastically increased in the last sample before local extinction (**Figures 6B**, **7B**, Supplementary Figure 3B), indicating diversified bet-hedging. These observations imply that specimens of *G. scitula* began to grow faster (i.e., adding larger chambers) shortly before local extinction whilst at the same time the intra-individual continuity of chamber size was significantly decreased. Moreover, while the increase in *IG* affected the whole community alike, the *IGV* showed a high inter-individual variance at the same time, implying that the observed reaction affected only part of the investigated community.

### STABILIZING AND DISRUPTIVE SELECTION AND BET-HEDGING IN PLANKTONIC FORAMINIFERA

#### *Stabilizing selection on shell size*

Haenel (1987) interpreted the size of shells of *O. universa* as an indicator of water salinity, arguing that the larger size counteracts buoyancy when the water becomes less dense due to a reduction in salinity. An alternative hypothesis correlates the size of the terminal chamber in *O. universa* with food availability (Spero, 1988), arguing that the energy needed to generate a larger terminal chamber can only be compensated by larger energy reserves within the cell. Under those assumptions, larger shells should have been observed within the sapropel, since sea water salinity was reduced (Van Der Meer et al., 2007) and the primary productivity was higher (Struck et al., 2001) during sapropel conditions.

Rather than such predicted changes in the mean shell size of the population, we observe a significant decrease in shell-size variability in *O. universa* after the onset of the sapropel deposition. We hypothesize, that the higher variability in shell size in *O. universa* before sapropel onset reflects disruptive selection, where a higher variability of the community is necessitated to sustain under sub-optimal conditions. The lack of a bimodal size-distribution at that time is here interpreted as a sign of a moderate disruption, and is also what one would expect with disruptive selection under random mating models that can arguably be assumed for Foraminifera (compare Doebeli, 1996). During sapropel conditions, on the other hand, the environmental niche of the species was narrowed, with more open-marine conditions (normal marine salinity) and high nutrition values of the sea water (Struck et al., 2001) that could better sustain a population with lower phenotypic plasticity due to higher resource availability for an abundant mean phenotype (Rueffler et al., 2006).

The local extinctions, however, did not leave any discernible imprint in the size distribution of either of the two species, rendering this trait useless for extinction prediction. Furthermore, this is evidence against the hypothesis, that larger shell sizes are correlated with favorable environmental conditions (Ortiz et al., 1995; Schmidt et al., 2004), because then we would expect to find smaller specimens at times of enhanced stress levels, such as prior to local extinctions.

#### *Fluctuating asymmetry as a proxy for stress*

The roundness of the terminal chamber in *O. universa* shows a significant reduction after sapropel onset, contemporaneously with a reduction in shell calcification which was hypothesized to reflect the influence of the reduced surface water salinity and resulting change in the $CO_3^{2-}$ equilibrium of the sea water after the onset of the sapropel (Weinkauf et al., 2013).

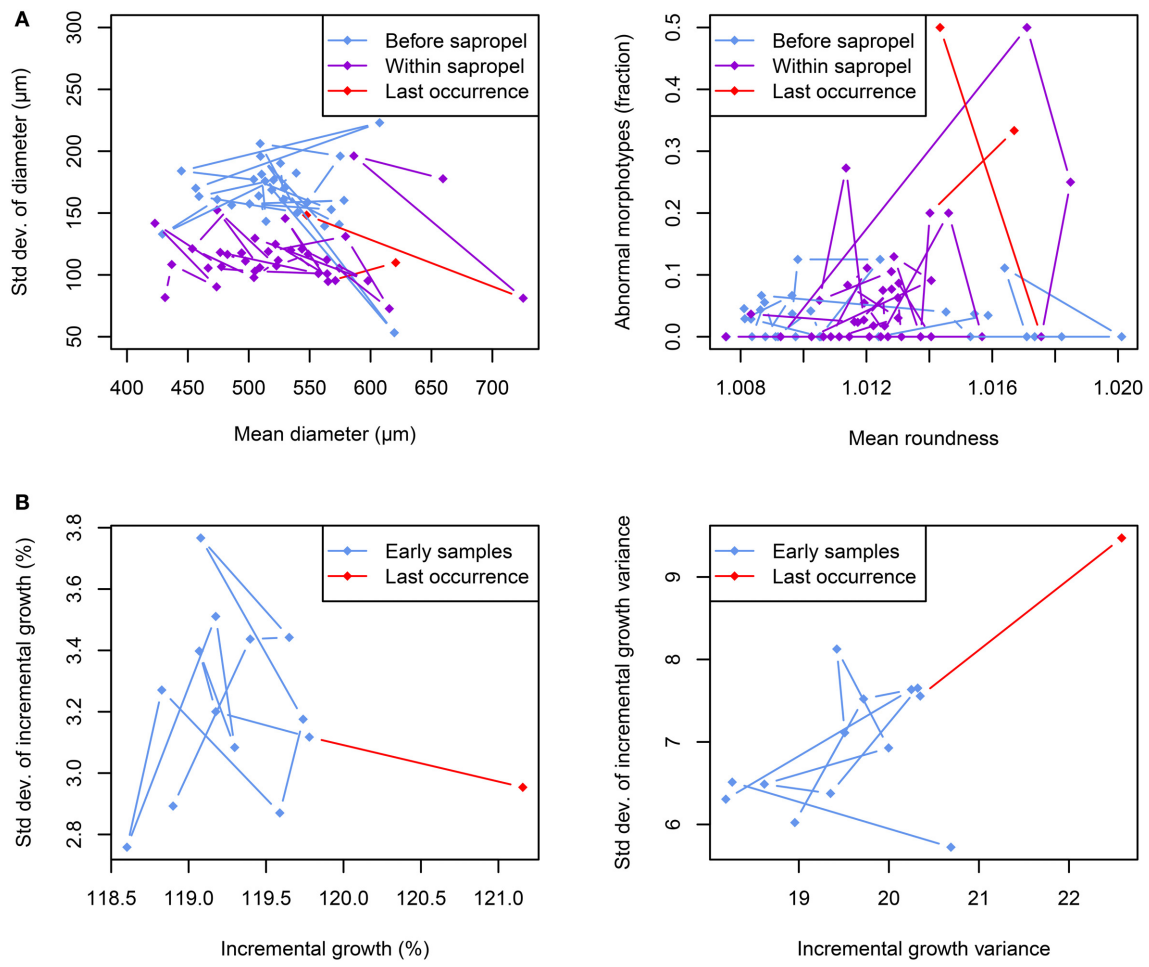



**FIGURE 7 | Morphological trends in foraminiferal shells during extinction periods. (A)** Evolution of size and roundness and incidence of abnormal morphotypes in *Orbulina universa* across the onset of the Sapropel S5 deposition in core M51-3/SL104. The shell diameter shows no change with sapropel onset, but size variability is reduced within the sapropel. In contrast, shells within the sapropel are on average significantly less round than for the majority of the time before sapropel onset. The incidence of abnormal morphotypes is generally higher within the sapropel than before, with peak abundances before local extinctions. **(B)** In *Globorotalia scitula* a change in the spiral growth pattern can be observed immediately prior to local extinction. While the mean value of incremental growth increases (indicating the building of larger chambers), its variance remains the same on the population level. However, the incremental growth variance within an individual becomes both larger and more variable on the population level.

Considering the fact that mean shell roundness is constantly low over long time intervals, but without exhibiting any deviation when the species approaches local extinction (**Figure 5A**), the reduction in roundness seems to have been induced by environmental parameters which are not themselves unfavorable for that species. It has been suggested that the shell roundness of *O. universa* decreases under conditions of high nutrient availability (Robbins, 1988; Spero, 1988), which can be assumed during sapropel deposition (Struck et al., 2001). The same factors were also argued to be responsible for higher incidences of abnormal morphotypes in *O. universa* (Robbins, 1988), what we can partly confirm on the basis of our results. However, besides a generally higher incidence of abnormal morphotypes within the sapropel we also found peak abundances of those types shortly before local extinctions, indicating that they can also be the result of increased environmental stress as suggested by Caron et al. (1987). A general increase of the abundance of phentotypes that are more rare under optimal conditions can be interpreted in the lines of conservative bet-hedging, that has been shown to generally increase the mean fitness of a population in variable environments (Einum and Fleming, 2004). Thus we suggest here that abnormal morphotypes in *O. universa* are an adaptive response of that species toward less suitable or more variable environmental conditions. This explains both the general higher incidence of abnormal morphotypes within the early sapropel stages investigated here, during which the severe overturn in water mass circulation likely led to increased environmental variability before the system re-stabilized, as well as the peak abundances shortly before local extinctions, when some environmental factors must have been especially unfavorable for *O. universa*.

The deviation of the spiral growth pattern in *G. scitula* toward the terminal environmental stress is more difficult to explain. On the one hand, we were able to observe changes in the chamber-by-chamber growth pattern. Under the assumption that the chamber

size is proportional to the degree of cytoplasm growth, faster proportional chamber growth indicates that more cytoplasm was produced between the formation of two successive chambers. This could on the one hand be the result of disturbances in the timing of chamber formation, building new chambers less often, so that more cytoplasm has been produced since. This explanation would probably indicate a lack of nutrients, where the timing of chamber formation participates in a trade-off with cellular energy resources. On the other hand, chamber formation could occur on strictly regular time intervals, in which case larger chambers would indicate larger cytoplasm growth per time as result of higher nutrient availability.

At the same time, however, we observe a decrease in the intra-individual evenness of chamber growth, indicating either a reduction in the ability of the cell to control the exact size of the chamber produced, or a more irregular time-pattern of chamber formation. Conversely, the ability of the cell to maintain its logarithmic spiral growth pattern is retained. Interestingly, the observed increase in *IGV* is itself subject to an increased inter-individual variability when the species approaches local extinction. This observation can be interpreted in at least two ways. (1) It is possible that the elevated level of environmental stress had not affected the entire interval of 60–70 yrs expected to be represented by the last sample before local extinction, and thus only the historically younger part of the community shows the described effect. We could not find a significant deviation from unimodality in the *IGV* data of *G. scitula* from the last sample before extinction ($p = 0.877$), principally arguing against that hypothesis. (2) The increase in *IGV* variability in *G. scitula* could also be interpreted as bet-hedging under high-stress environmental conditions. In this case, we would expect higher variability of *IGV* on the community level as a result of higher phenotypic plasticity. This phenomenon would represent a case of diversified bet-hedging (Einum and Fleming, 2004), where the overall variability of the population is increased in order to allow the survival of at least some offspring. Einum and Fleming (2004) suggested that diversified bet-hedging can only be of advantage for the population if the environment is very unstable, and the population is practically at the brink of extinction. Interestingly enough, the local extinction in *G. scitula* occurs contemporaneously with the onset of Sapropel S5, which led to heavy changes in the Eastern Mediterranean vertical circulation system. Accordingly, since a marine water-mass circulation cannot be changed spontaneously, it is reasonable to assume that such heavily variable environments prevailed at that time, especially at greater depths where *G. scitula* dwells, that are dependent on the supply by surface waters to remain environmentally stable. Such an interpretation could also explain why *O. universa* as a surface dweller (where the environmental change mainly consisted of a gradual change in salinity) showed conservative bet-hedging with sapropel onset, and why *G. scitula* was able to reinvade the Eastern Mediterranean after the sapropel conditions had been established (**Figure 3**) and the deeper water column was also stable again.

In conclusion, we suggest that there are two types of FA realized in Foraminifera. Such a distinction seems to be necessary to accommodate our results, and is reasonable due to the fact that the concept of FA was originally developed for bilateral multicellular organisms and is not applicable to protists in this narrow form. FA s.lat. represents the overall shape of the foraminifer shell, such as the roundness of *O. universa* shells or the logarithmic spiral of shells of *G. scitula*. This type of FA is robust against environmental stress (although it seems to be influenced by other environmental parameters) and is thus not suitable as proxy for local extinctions.

FA s.str. is here defined as the ability of a foraminifer to constrain the chamber by chamber growth pattern of its shell, and includes the incidence of abnormal morphotypes in *O. universa* and *IG* and *IGV* in *G. scitula*. Many of the abnormal specimens of benthic Foraminifera described as the result of water pollution seem to be more extreme forms of this FA s.str. (Alve, 1991; Burone et al., 2006). This form of FA thus seems to be the result of bet-hedging occurring under high stress conditions, providing a versatile tool to predict extinctions (Leung et al., 2000; Sánchez-Chardi et al., 2013).

#### PREDICTABILITY OF EXTINCTION FROM PHENOTYPE HISTORY

We could observe both long-term and short-term reactions in shell morphology of planktonic Foraminifera and can thus verify our initial hypothesis. Long-term changes in trait state result in constant trait-states over thousands of years and are associated with prevailing environmental shifts inducing certain morphotypes, and can under circumstances lead to stabilizing selection, reducing the variance in shell morphology within the population. We can interpret this as a signal for stable, possibly favorable environmental conditions, under circumstances with reduced stress levels. Short-term changes occur relatively abrupt and prevail for only decades or few centuries, and are the result of environmental stress reactions of the population toward a relatively sudden environmental shift, often associated with unstable environments.

Disruptive selection, on the other hand, leads to an increase in variance within the community, and can be observed on both short-term and long-term scales in planktonic Foraminifera. We hypothesize that disruptive selection is the imprint of a stress reaction toward unfavorable environments. The observed disruption can be interpreted as the possible result of bet-hedging, a process that increases the mean fitness of the population (Philippi and Seger, 1989). Our analysis shows, that bet-hedging can sometimes not prevent extinction, but can otherwise lead to a measurable increase in variance of the population over considerable time intervals. While bet-hedging as a phenomenon prevails in unicellular organisms (Veening et al., 2008) and has been hypothesized to be one of the most fundamental survival strategies (Beaumont et al., 2009), it has not been shown to occur in planktonic Foraminifera yet, and has thus never been considered as an environmental proxy so far.

We thus contribute to other studies by showing that many of the selective patterns observed in phylogenetically more advanced organisms occur in protists. Some of these mechanisms (bet-hedging) leave a discernable imprint of a unique population trait composition (FA s.str.) that can be used to predict local extinctions. While the overall architecture of the shells is very robust against environmental perturbations, the incremental growth pattern of foraminiferal shells (representing FA s.str.) can be a valuable proxy for environmental stress levels, given that the stress

levels were raised over decades (100s of generations) so that the proportion of affected individuals in the sample is large enough to be detected.

## ACKNOWLEDGMENTS

We want to thank Richard Telford (University of Bergen) for critical comments on the results of our study. Norman MacLeod (Natural History Museum London) is acknowledged for helpful comments on an earlier version of this manuscript. Further acknowledgment goes to the other members of the Micropaleontology work group in Bremen and the Minigraduiertenkolleg in Tübingen, whose contributions cannot be listed individually here. Manuel F. G. Weinkauf was supported by the LGFG Baden–Württemberg and the Johanna M. Resig Fellowship of the Cushman Foundation. Tobias Moller received funding from the German Research Foundation DFG-grant SCHU1605/3-1.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fevo.2014.00064/abstract

All morphometric raw data associated with this work are available on PANGAEA (doi: 10.1594/PANGAEA.832132).

## REFERENCES

Alve, E. (1991). Benthic foraminifera in sediment cores reflecting heavy metal pollution in Sørfjord, western Norway. *J. Foraminiferal Res.* 21, 1–19. doi: 10.2113/gsjfr.21.1.1

André, A., Weiner, A., Quillévéré, F., Aurahs, R., Morard, R., Douady, C. J., et al. (2013). The cryptic and the apparent reversed: lack of genetic differentiation within the morphologically diverse plexus of the planktonic foraminifer *Globigerinoides sacculifer*. *Paleobiology* 39, 21–39. doi: 10.1666/0094-8373-39.1.21

Aurahs, R., Treis, Y., Darling, K. F., and Kučera, M. (2011). A revised taxonomic and phylogenetic concept for the planktonic foraminifer species *Globigerinoides ruber* based on molecular and morphometric evidence. *Mar. Micropaleontol.* 79, 1–14. doi: 10.1016/j.marmicro.2010.12.001

Beasley, D. A. E., Bonisoli-Alquati, A., and Mousseau, T. A. (2013). The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: a meta-analysis. *Ecol. Indic.* 30, 218–226. doi: 10.1016/j.ecolind.2013.02.024

Beaumont, H. J. E., Gallie, J., Kost, C., Ferguson, G. C., and Rainey, P. B. (2009). Experimental evolution of bet hedging. *Nature* 462, 90–93. doi: 10.1038/nature08504

Bé, A. W. H., Hemleben, C., Anderson, O. R., and Spindler, M. (1979). Chamber formation in planktonic foraminifera. *Micropaleontology* 25, 294–307. doi: 10.2307/1485304

Bull, J. J. (1987). Evolution of phenotypic variance. *Evol. Int. J. Org. Evol.* 41, 303–315. doi: 10.2307/2409140

Burone, L., Venturini, N., Sprechmann, P., Valente, P., and Muniz, P. (2006). Foraminiferal responses to polluted sediments in the Montevideo coastal zone, Uruguay. *Mar. Pollut. Bull.* 52, 61–73. doi: 10.1016/j.marpolbul.2005.08.007

Cane, T., Rohling, E. J., Kemp, A. E. S., Cooke, S., and Pearce, R. B. (2002). High-resolution stratigraphic framework for Mediterranean Sapropel S5: defining temporal relationships between records of Eemian climate variability. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 183, 87–101. doi: 10.1016/S0031-0182(01)00461-8

Caron, D. A., Faber, J. W. F., and Bé, A. W. H. (1987). Growth of the spinose planktonic foraminifer *Orbulina universa* in laboratory culture and the effect of temperature on life processes. *J. Mar. Biol. Assoc. U.K.* 67, 343–358. doi: 10.1017/S0025315400026655

Davison, A. C., and Hinkley, D. V. (1997). *Bootstrap Methods and their Applications.* Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511802843

Debat, V., Milton, C. C., Rutherford, S., Klingenberg, C. P., and Hoffmann, A. A. (2006). Hsp90 and the quantitative variation of wing shape in *Drosophila melanogaster*. *Evol. Int. J. Org. Evol.* 60, 2529–2538. doi: 10.1554/06-045.1

Delord, J. (2007). The nature of extinction. *Stud. Hist. Philos. Sci.* 38, 656–667. doi: 10.1016/j.shpsc.2007.06.004

De Vargas, C., Norris, R. D., Zaninetti, L., Gibb, S. W., and Pawlowski, J. (1999). Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces. *Proc. Natl. Acad. Sci. U.S.A.* 96, 2864–2868. doi: 10.1073/pnas.96.6.2864

Dixon, P. M. (2002). "Bootstrap resampling," in *Encyclopedia of Environmetrics*, eds A. H. El-Shaarawi and W. W. Piegorsch (Chichester: John Wiley & Sons, Ltd.), 212–220.

Doebeli, M. (1996). A quantitative genetic competition model for sympatric speciation. *J. Evol. Biol.* 9, 893–909. doi: 10.1046/j.1420-9101.1996.9060893.x

Drake, J. M., and Griffen, B. D. (2010). Early warning signals of extinction in deteriorating environments. *Nature* 467, 456–459. doi: 10.1038/nature09389

Einum, S., and Fleming, I. A. (1999). Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proc. Biol. Sci.* 266, 2095–2100. doi: 10.1098/rspb.1999.0893

Einum, S., and Fleming, I. A. (2004). Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. *Evol. Ecol. Res.* 6, 443–455.

Furlow, F. B., Armijo-Prewitt, T., Gangestad, S. W., and Thornhill, R. (1997). Fluctuating asymmetry and psychometric intelligence. *Proc. Biol. Sci.* 264, 823–829. doi: 10.1098/rspb.1997.0115

Grafen, A. (1999). Formal Darwinism, the individual-as-maximizing-agent analogy and bet-hedging. *Proc. Biol. Sci.* 266, 799–803. doi: 10.1098/rspb.1999.0708

Grubbs, F. E. (1969). Procedures for detecting outlying observations in samples. *Technometrics* 11, 1–21. doi: 10.1080/00401706.1969.10490657

Haenel, P. (1987). Intérêt paléoocéanographique d'*Orbulina universa* d'Orbigny (foraminifère). *Oceanol. Acta* 10, 15–25.

Hartigan, J. A., and Hartigan, P. M. (1985). The dip test of unimodality. *Ann. Stat.* 13, 70–84. doi: 10.1214/aos/1176346577

Hemleben, C., Becker, T., Bellas, S., Benningsen, G., Casford, J., Cagatay, N., et al. (2003). *Ostatlantik–Mittelmeer–Schwarzes Meer Part 3: Cruise No. 51, Leg 3.* Hamburg: Leitstelle METEOR.

Hendrickx, F., Maelfait, J. P., and Lens, L. (2003). Relationship between fluctuating asymmetry and fitness within and between stressed and unstressed populations of the wolf spider *Pirata piraticus*. *J. Evol. Biol.* 16, 1270–1279. doi: 10.1046/j.1420-9101.2003.00633.x

Heslop, D., De Schepper, S., and Proske, U. (2011). Diagnosing the uncertainty of taxa relative abundances derived from count data. *Mar. Micropaleontol.* 79, 114–120. doi: 10.1016/j.marmicro.2011.01.007

Kučera, M. (2007). "Planktonic Foraminifera as tracers of past oceanic environments," in *Proxies in Late Cenozoic Paleoceanography*, eds C. Hillaire-Marcel, A. De Vernal, and H. Chamley (Amsterdam: Elsevier), 213–262. doi: 10.1016/S1572-5480(07)01011-1

Legendre, P., and Legendre, L. (2012). *Numerical Ecology.* Amsterdam; Oxford: Elsevier.

Lens, L., Van Dongen, S., Kark, S., and Matthysen, E. (2002). Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? *Biol. Rev. Camb. Philos. Soc.* 77, 27–38. doi: 10.1017/S1464793101005796

Leung, B., Forbes, M. R., and Houle, D. (2000). Fluctuating asymmetry as a bioindicator of stress: comparing efficacy of analyses involving multiple traits. *Am. Nat.* 155, 101–115. doi: 10.1086/303298

Ludwig, D. (1999). Is it meaningful to estimate a probability of extinction? *Ecology* 80, 298–310. doi: 10.1890/0012-9658(1999)080[0298:IIMTEA]2.0.CO;2

Malmgren, B. A., and Kennett, J. P. (1976). Biometric analysis of phenotypic variation in recent *Globigerina bulloides* d'Orbigny in the southern Indian Ocean. *Mar. Micropaleontol.* 1, 3–25. doi: 10.1016/0377-8398(76)90003-7

Mann, H. B., and Whitney, D. R. (1947). On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Stat.* 18, 50–60. doi: 10.1214/aoms/1177730491

Marino, G., Rohling, E. J., Rijpstra, W. I. C., Sangiorgi, F., Schouten, S., and Damsté, J. S. S. (2007). Aegean Sea as driver of hydrographic and ecological changes in the Eastern Mediterranean. *Geology* 35, 675–678. doi: 10.1130/G23831A.1

Mary, Y., and Knappertsbusch, M. W. (2013). Morphological variability of menardiform globorotalids in the Atlantic Ocean during Mid-Pliocene. *Mar. Micropaleontol.* 101, 180–193. doi: 10.1016/j.marmicro.2012.12.001

Moller, T. (2012). *Formation and Palaeoclimatic interpretation of a Continuously Laminated Sapropel S5: A Window to the Climate Variability During the Eemian Interglacial in the Eastern Mediterranean*. Ph.D. thesis, Eberhard–Karls Universität Tübingen, Tübingen.

Moller, T., Schulz, H., Hamann, Y., Dellwig, O., and Kučera, M. (2012). Sedimentology and geochemistry of an exceptionally preserved last interglacial Sapropel S5 in the Levantine Basin (Mediterranean Sea). *Mar. Geol.* 291–294, 34–48. doi: 10.1016/j.margeo.2011.10.011

Moritz, C., and Agudo, R. (2013). The future of species under climate change: resilience or decline? *Science* 341, 504–508. doi: 10.1126/science.1237190

Ortiz, J. D., Mix, A. C., and Collier, R. W. (1995). Environmental control of living symbiotic and asymbiotic Foraminifera of the California Current. *Paleoceanography* 10, 987–1009. doi: 10.1029/95PA02088

Pélabon, C., Hansen, T. F., Carter, A. J. R., and Houle, D. (2010). Evolution of variation and variability under fluctuating, stabilizing, and disruptive selection. *Evol. Int. J. Org. Evol.* 64, 1912–1925. doi: 10.1111/j.1558-5646.2010.00979.x

Philippi, T., and Seger, J. (1989). Hedging one's evolutionary bets, revisited. *Trends Ecol. Evol.* 4, 41–44. doi: 10.1016/0169-5347(89)90138-9

Pujol, C., and Vergnaud Grazzini, C. (1995). Distribution patterns of live planktic foraminifers as related to regional hydrography and productive systems of the Mediterranean Sea. *Mar. Micropaleontol.* 25, 187–217. doi: 10.1016/0377-8398(95)00002-I

R Development Core Team. (2013). *R: a Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Robbins, L. L. (1988). Environmental significance of morphologic variability in open-ocean versus ocean-margin assemblages of *Orbulina universa*. *J. Foraminiferal Res.* 18, 326–333. doi: 10.2113/gsjfr.18.4.326

Rohling, E., Abu-Zied, R., Casford, J., Hayes, A., and Hoogakker, B. (2009). "The marine environment: present and past," in *The Physical Geography of the Mediterranean,* ed J. Woodward (New York, NY: Oxford University Press), 33–67.

Rohling, E. J. (1994). Review and new aspects concerning the formation of Eastern Mediterranean sapropels. *Mar. Geol.* 122, 1–28. doi: 10.1016/0025-3227(94)90202-X

Rohling, E. J., Sprovieri, M., Cane, T., Casford, J. S. L., Cooke, S., Bouloubassi, I., et al. (2004). Reconstructing past planktic foraminiferal habitats using stable isotope data: a case history for Mediterranean Sapropel S5. *Mar. Micropaleontol.* 50, 89–123. doi: 10.1016/S0377-8398(03)00068-9

Rossignol-Strick, M., Nesteroff, W., Olive, P., and Vergnaud Grazzini, C. (1982). After the deluge: mediterranean stagnation and sapropel formation. *Nature* 295, 105–110. doi: 10.1038/295105a0

Rueffler, C., Van Dooren, T. J. M., Leimar, O., and Abrams, P. A. (2006). Disruptive selection and then what? *Trends Ecol. Evol.* 21, 238–245. doi: 10.1016/j.tree.2006.03.003

Sánchez-Chardi, A., García-Pando, M., and López-Fuster, M. J. (2013). Chronic exposure to environmental stressors induces fluctuating asymmetry in shrews inhabiting protected Mediterranean sites. *Chemosphere* 93, 916–923. doi: 10.1016/j.chemosphere.2013.05.056

Schiebel, R., Waniek, J., Zeltner, A., and Alves, M. (2002). Impact of the Azores front on the distribution of planktic foraminifers, shelled gastropods, and coccolithophorids. *Deep Sea Res. II* 49, 4035–4050. doi: 10.1016/S0967-0645(02)00141-8

Schmidt, D. N., Renaud, S., Bollmann, J., Schiebel, R., and Thierstein, H. R. (2004). Size distribution of Holocene planktic foraminifer assemblages: biogeography, ecology and adaptation. *Mar. Micropaleontol.* 50, 319–338. doi: 10.1016/S0377-8398(03)00098-7

Shapiro, S. S., and Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika* 52, 591–611. doi: 10.1093/biomet/52.3-4.591

Sheskin, D. J. (2011). *Handbook of Parametric and Nonparametric Statistical Procedures.* Boca Raton; New York: Chapman & Hall/CRC Press.

Slatkin, M. (1974). Hedging one's evolutionary bets. *Nature* 250, 704–705. doi: 10.1038/250704b0

Spero, H. J. (1988). Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*. *Mar. Biol.* 99, 9–20. doi: 10.1007/BF00644972

Spero, H. J., Lerche, I., and Williams, D. F. (1991). Opening the carbon isotope "Vital Effect" black box, 2, quantitative model for interpreting foraminiferal carbon isotope data. *Paleoceanography* 6, 639–655. doi: 10.1029/91PA02022

Struck, U., Emeis, K.-C., Voß, M., Krom, M. D., and Rau, G. H. (2001). Biological productivity during Sapropel S5 formation in the Eastern Mediterranean Sea: evidence from stable isotopes of nitrogen and carbon. *Geochim. Cosmochim. Acta* 65, 3249–3266. doi: 10.1016/S0016-7037(01)00668-8

Tabachnick, B. G., and Fidell, L. S. (1996). *Using Multivariate Statistics.* New York, NY: Harper Collins.

Tabor, J. (2010). Investigating the investigative task: testing for skewness—an investigation of different test statistics and their power to detect skewness. *J. Stat. Educ.* 18, 1–13. http://www.amstat.org/publications/jse/v18n2/tabor.pdf

Van Der Meer, M. T. J., Baas, M., Rijpstra, W. I. C., Marino, G., Rohling, E. J., Damsté, J. S. S., et al. (2007). Hydrogen isotopic compositions of long-chain alkenones record freshwater flooding of the Eastern Mediterranean at the onset of sapropel deposition. *Earth Planet. Sci. Lett.* 262, 594–600. doi: 10.1016/j.epsl.2007.08.014

Van Valen, L. (1965). Morphological variation and width of ecological niche. *Am. Nat.* 99, 377–390. doi: 10.1086/282379

Veening, J.-W., Smits, W. K., and Kuipers, O. P. (2008). Bistability, epigenetics, and bet-hedging in bacteria. *Annu. Rev. Microbiol.* 62, 193–210. doi: 10.1146/annurev.micro.62.081307.163002

Weinkauf, M. F. G., Moller, T., Koch, M. C., and Kučera, M. (2013). Calcification intensity in planktonic foraminifera reflects ambient conditions irrespective of environmental stress. *Biogeosciences* 10, 6639–6655. doi: 10.5194/bg-10-6639-2013

Williams, D. F., Thunell, R. C., and Kennett, J. P. (1978). Periodic freshwater flooding and stagnation of the Eastern Mediterranean Sea during the late Quaternary. *Science* 201, 252–254. doi: 10.1126/science.201.4352.252

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

*Received: 12 August 2014; accepted: 24 September 2014; published online: 14 October 2014.*

*Citation: Weinkauf MFG, Moller T, Koch MC and Kučera M (2014) Disruptive selection and bet-hedging in planktonic Foraminifera: shell morphology as predictor of extinctions. Front. Ecol. Evol.* ***2****:64. doi: 10.3389/fevo.2014.00064*

*This article was submitted to Paleoecology, a section of the journal Frontiers in Ecology and Evolution.*

# Chapter 7

**Manuscript 4**: Weinkauf, M. F. G., Braun, K., Bonitz, F., and Kučera, M. (in preparation) Stabilization and disruption as indicator of terminal stress and extinction in planktonic Foraminifera, *PLOS ONE*

## Abstract

The effects of Environmental stress, potentially on a terminal level leading to extinction, are remarkably difficult to assess, because their recognition in recent environments is complicated. Approaches using population dynamics as proxy for stress suffer from the problem of a naturally large variability of population sizes, so that morphometrics has been developed as an alternative approach for stress assessment. In this study, we use morphometric approaches to quantify morphological change in a planktonic Foraminifera community that was exposed to salinity-induced terminal stress levels during Marine Isotope Stage 12 in the Red Sea. We find disparities in the reaction norm of the two species studied: while *Orbulina universa* responds by morphological change consistent with disruptive selection, *Globigerinoides sacculifer* exhibits multilevel microenvironmental canalization and stabilizing selection during the same time. Both species were tested for a correlation between morphology, and sea water salinity and species abundance. Shell morphology and phenotypic plasticity seem to reflect environmental stress patterns. However, the abiotic forcing by the salinity change cannot be fully disentangled from the biotic stress reactions. Both species exhibit morphological gradients in correlation with salinity, that serve as a better indicator for environmental stress on the community than the species abundance. We could therefore show that morphometric analyses in planktonic Foraminifera are a versatile tool to predict past stress levels and impending extinction, but that reaction norms are species specific and further complicated by biological integration.

# Stabilization and disruption as indicator of terminal stress and extinction in planktonic Foraminifera

**Manuel F. G. Weinkauf[1 2] *, Kerstin Braun[1 #a], Fabian Bonitz[1 #b], Michal Kučera[2]**

**1** Department of Geosciences, Eberhard–Karls Universität, Tübingen, Germany, **2** MARUM, University Bremen, Bremen, Germany, **#a** Current address: Institute of Human Origins, School of Human Evolution and Social Change, Arizona State University, Tempe, USA, **#b** Current address: Bjerknes Centre for Climate Research, Universitetet i Bergen, Bergen, Norway

* E-mail: mweinkauf@marum.de

## Abstract

The effects of Environmental stress, potentially on a terminal level leading to extinction, are remarkably difficult to assess, because their recognition in recent environments is complicated. Approaches using population dynamics as proxy for stress suffer from the problem of a naturally large variability of population sizes, so that morphometrics has been developed as an alternative approach for stress assessment. In this study, we use morphometric approaches to quantify morphological change in a planktonic Foraminifera community that was exposed to salinity-induced terminal stress levels during Marine Isotope Stage 12 in the Red Sea. We find disparities in the reaction norm of the two species studied: while *Orbulina universa* responds by morphological change consistent with disruptive selection, *Globigerinoides sacculifer* exhibits multilevel microenvironmental canalization and stabilizing selection during the same time. Both species were tested for a correlation between morphology, and sea water salinity and species abundance. Shell morphology and phenotypic plasticity seem to reflect environmental stress patterns. However, the abiotic forcing by the salinity change cannot be fully disentangled from the biotic stress reactions. Both species exhibit morphological gradients in correlation with salinity, that serve as a better indicator for environmental stress on the community than the species abundance. We could therefore show that morphometric analyses in planktonic Foraminifera are a versatile tool to predict past stress levels and impending extinction, but that reaction norms are species specific and further complicated by biological integration.

**Funding:** Ministerium für Wissenschaft, Forschung und Kunst Baden–Württemberg (LGFG stipend), Johanna M. Resig Foraminifera Research Fellowship (The Cushman Foundation for Foraminiferal Research). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Introduction

Extinction events are remarkably difficult to study, mainly because it is complicated to foresee extinctions in recent environments [1]. While large efforts have been made to predict extinctions via population dynamics analyses [2] this approach is still suffering from the naturally large variability of population sizes [3]. Morphometrics could therefore serve as an alternative (and independent) tool for predicting stress levels and identifying impending extinctions.

Shape and size have long been hypothesized to reflect the influence of environmental stress on the physiology of an organism during its lifetime [4-10]. Therefore, a characterisation of shape and size and their variance should in principle allow an assessment of the severity of stress exposure. Under this assumption, stress exposure that leads to extinction can be expected to leave a discernible imprint on morphology in pre-extinction populations.

Developmental stability [11], as inferred from morphology, has been shown to be influenced by environmental stress [4, 5, 12], which is here defined as the sum of all biotic and abiotic factors that deviate from the optimum requirements of a species. Mechanistically, environmental stress influences population morphology by decreasing the fitness of specimens which show a high degree of developmental instability [6, 7]. On the population level, this can lead to either stabilizing selection, i.e. a narrowing of the reaction norm [13], or disruptive selection, the broadening of the reaction norm [14]. Both stabilizing and disruptive selection are detectable in the population by assessing the phenotypic plasticity (i.e. the range of realized phenotypes [15]) of the community, which can therefore be used as a measure for developmental stability.

While studies on developmental stability are frequently found in other organisms [8, 12, 16-19] there is still much controversy as to what extent population morphology truly reflects the stress levels a community is exposed to [20, 21]. Partly, this is because it is difficult to study stress reactions over ecologically relevant timescales, which cannot be simulated in the laboratory, or because the severity and ultimate outcome of the stress reaction is hard to predict.

In this regard, the sedimentary record offers a unique opportunity to study the effects of terminal stress levels (i.e. stress leading to extinction), because here the local extinctions can be directly observed and the place and interval for study can be chosen appropriately. This benefit comes at the cost that the sedimentary record always comprises a temporally integrated sequence where each sample includes several generations. Furthermore, the environmental change that caused the local extinction can be hard to reconstruct, and can in many cases even be an interplay between changes in several parameters that occur at the same time [22].

In this study, we therefore make use of a sedimentary record from the Red Sea, where several species of planktonic Foraminifera regionally disappeared from the fossil record (hereafter called local extinction) as a result of environmental change [23]. Calcitic marine microplankton, such as planktonic Foraminifera, exhibit high preservation potential and occur in large abundances in marine sediments [24]. They are therefore a perfect model group to assess the morphological reaction of organisms to terminal stress levels. Furthermore, in contrast to comparable cases where fossil material is used, we are here in the unique situation, that the environmental gradients are known and the extinction events can nearly exclusively be linked to salinity increase [25].

Understanding the morphological reaction of Foraminifera towards environmental stress could serve as both a proxy for environmental reconstructions and also further our understanding of evolvability of this organismal group. Past studies of Foraminifera morphology have shown, that morphological deviations in planktonic Foraminifera can be caused by environmental forcing [22, 26-29]. But since those studies are few and quantified morphology in very different ways, the results obtained are scarce and controversial. Given results by those earlier studies it is reasonable to assume to see a morphological trend in the community of planktonic Foraminifera associated with sea level and thus salinity changes and the resulting terminal stress levels. Our hypothesis is therefore to find a correlation between morphology and either sea level (as indicator of the abiotic factor salinity) or species abundance (as biotic indicator for the stress level the community is exposed to). This understanding is fundamentally important, because it would help to disentangle environmental forcing of morphology from pure stress-reactions. Should the environmental factor play a dominant role in organism morphology, regardless of the amount of stress it introduces, then morphology should mainly change as a function of salinity. Should the stress-level a community is exposed to be the dominant factor inducing morphological deviations we would assume to see a closer relationship between morphology and abundance patterns, under the assumption that

abundance is a good indicator for optimal growth conditions.

## Material and Methods

### Choice of sampling interval and species

For the present study we used material from piston core Geo-TÜ KL09 (19.804° N, 38.103° E, Figure 1) taken during the RV Meteor cruise M5-2 [30]. This core has an excellent record of isotopic data [25] so that the stress-gradient induced by salinity changes and the onset of the aplanktonic zone can be reliably reconstructed.

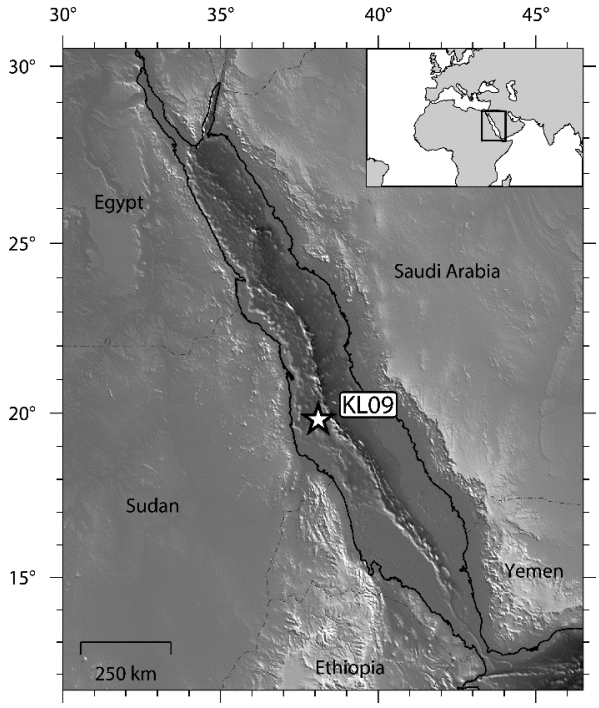



**Figure 1. Map of the sampling region.** The location of piston core Geo-TÜ KL09 in the Red Sea, off the coast of Sudan, is indicated by the white star. The region of the detailed map is indicated as rectangle in the small inlay map. Topographical information are based on the ETOPO1 dataset [31].

We chose material from MIS 12, corresponding to a time interval of 479.7–463.1 ka according to the age model by Rohling, Grant, Bolshaw et al. [25], to investigate the morphological reaction of planktonic Foraminifera to salinity-induced environmental stress leading to local extinction. The interval covers one of several aplanktonic zones that occurred during the Pleistocene in the Red Sea as a result of extremely high salinities (> 49 psu) induced by a changed circulation pattern in the Red Sea Basin [23]. Shortly before the onset of each aplanktonic zone, a distinct sequential extinction pattern of different planktonic Foraminifera species can be observed [23], until virtually all planktonic Foraminifera are absent from the fossil record. This particular aplanktonic zone has been chosen, because it is the most prominent and longest one in KL09 [23]. The spatial sampling resolution for our study varies between 0.5 cm and 2 cm such that a homogenous temporal resolution of 200–280 years/sample is achieved. Since salinity in the Red Sea is tightly coupled with the relative sea level [32], and high resolution sea level reconstructions from the Red Sea exist [25], we can approximate past sea water salinity to test for its influence on foraminiferal shell morphology specifically. While MIS 12 represents a glacial period, and a certain drop in sea surface temperature (SST) could therefore be assumed, other studies have shown that the SST in the Red Sea area rarely dropped below 24 °C during glacials [33], which is well within the normal temperature tolerance levels of all species common in the Red Sea [34, 35], so that sea surface temperature should have only played a minor role on environmental stress levels.

We have chosen to study the morphology of two symbiont-bearing species of planktonic Foraminifera, both of which occur in abundances high enough to be statistically interpretable. Both species react sensitively to salinity changes, as shown by their consistently early position within the extinction sequence of all aplanktonic zones in the Red Sea [23]. *Orbulina universa* is characterized by a trochospiral juvenile shell that in the adult stage is overgrown by a spherical terminal chamber. *Globigerinoides sacculifer* in contrast shows a trochospiral shell, in which the terminal chamber sometimes develops a sac-like shape. Both species are surface dwellers, partly due to their symbionts, and thus occur in comparable environments. Differences in their reactions can therefore not be the result of the exposure to different environmental forcing.

## Sample preparation and data acquisition

Samples of 0.5 cm thickness have been taken with a U-channel, dried, soaked in tap-water, and washed over a 63 µm screen under flowing tap-water. The residual > 63 µm was dried and dry-sieved over a 150 µm screen. Only the fraction > 150 µm was used for this study in order to ensure that the analysed individuals would have reached their adult stage so that an ontogenetic effect on the shape analyses could be eliminated.

For census counts only a small fraction of the samples (split with a microsplitter) has been used. Aliquots containing at least 300 specimens were investigated for their species composition and counted samples were transferred back into the glass vessels without modification. For morphological analyses specimens from representative aliquots (split with a microsplitter) were picked with a needle and transferred onto glass slides, were they were fixed in position using permanent glue. We were striving for a sample size of at least 50 specimens per sample for morphological analyses and in *O. universa* we were always using the whole sample aliquot. Since the manual data extraction in *G. sacculifer* was much more time-consuming we only used a randomly chosen subsample of 50 specimens/sample in that species, even if the picked aliquot contained more individuals.

Images for morphological analyses have been taken with a Canon EOS 500D digital mirror reflex camera attached to a Zeiss Stereo.V8 binocular microscope. To keep the measurement error for size-parameters constant we used a constant magnification per species for image capture.

Morphological data for *O. universa* (2775 specimens) were semi-automatically extracted from high-contrast transmitted light images using the software FIJI (ImageJ v. 1.49e [36]). In this species the shell size (as Feret diameter) and shell roundness (as ratio between longest and shortest axis of a fitted ellipse) have been extracted (Figure 2a). Using the axes of an ellipse fitted to the shell, instead of the min. and max. diameters of the shell ensures that small imperfections during data extraction (e.g. dirt on the shell surface) do not dominate the parameter. Furthermore, the incidence of the abnormal ecophenotypes '*Orbulina suturalis*' and '*Biorbulina bilobata*' [37] has been recorded.

In *G. sacculifer* (2230 specimens), images were taken under reflected light with specimens oriented such that the apertural plane was lying horizontally, perpendicular to the direction of view. From those images a total of 12 landmark points (Figure 2b, Table 1) were manually digitized in R v. 3.1.0 [38]. In some specimens parts of the structures were not visible clearly enough to extract all landmarks and those specimens (68) were excluded from all analyses using the landmark data. Furthermore, the coiling direction and attribution to one of the three morphotypes of the species (trilobus, quadrilobatus, sacculifer; compare André, Weiner, Quillévéré et al. [39]) has been recorded.

## Morphological data analysis

All statistical analyses have been performed in R v. 3.1.0. Where necessary to decide between the applicability of parametric or non-parametric tests the normality of data distribution was tested with a Shapiro–Wilk test [40] and the homoscedasticity by a Fligner–Killeen test [41]. Confidence intervals of morphological parameters were calculated via bootstrapping with the R-package 'boot' v. 1.3-11 [42]. We used basic bootstrapping when the data showed a skewness [43, equation I, table 1] that was higher than could be expected by chance [44] and accelerated bootstrapping otherwise [45]. Confidence intervals for the occurrences of morphotypes were calculated using multinomial equations [46]. Confidence intervals for standard deviations of morphological parameters were calculated following equations in Sheskin [47].

Morphological differences between groups for traditional morphometrics were investigated by a Kruskal–Wallis test [48], under circumstances followed by pairwise Mann–Whitney *U* tests [49]. In all cases of multiple testing (e.g. pairwise tests between more than two groups), *p*-values were corrected for the false discovery rate after Benjamini and Hochberg [50].

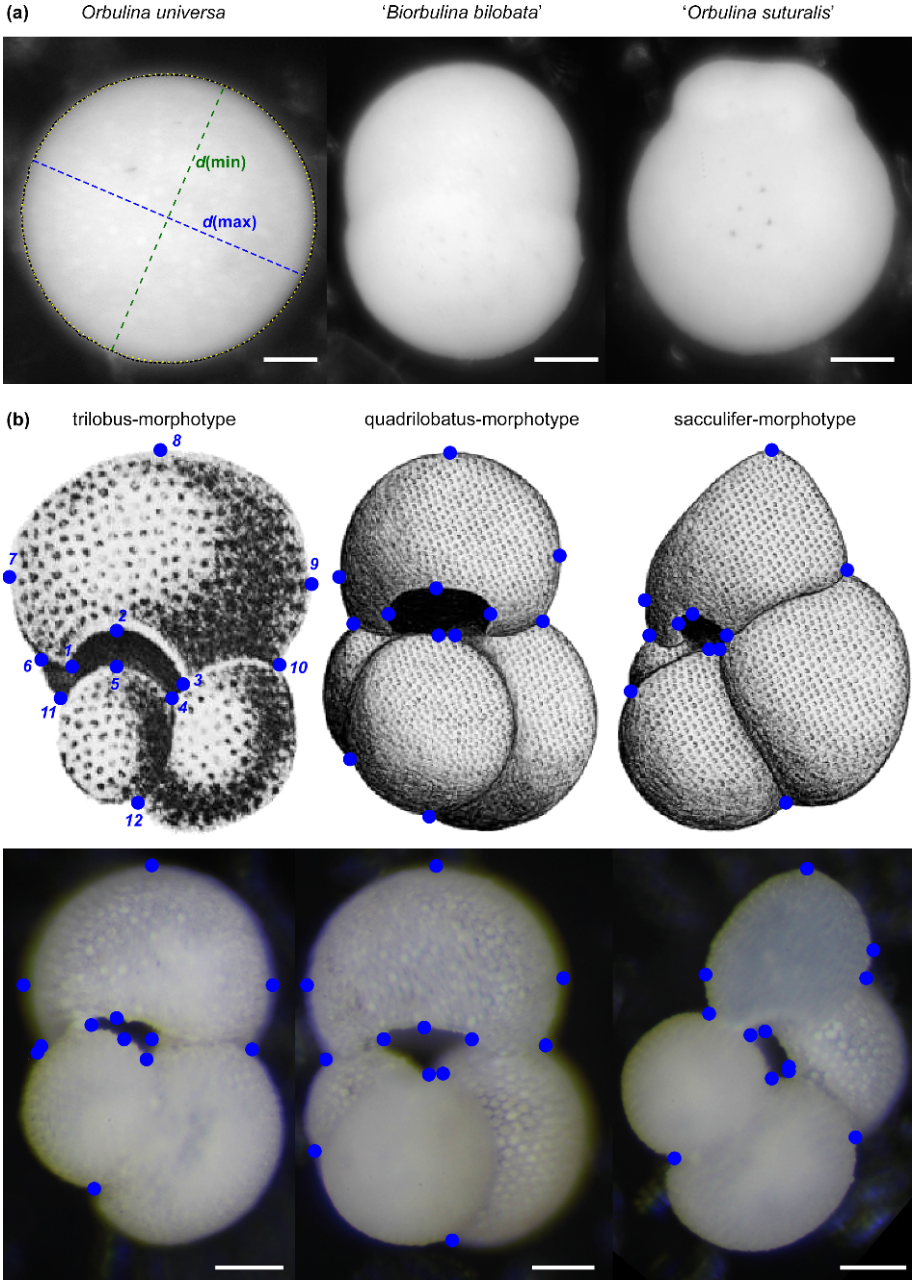



**Figure 2. Depiction of species and measurements. (a)** Measurements and morphotypes in *Orbulina universa*. The shell size was extracted as Feret diameter (not shown) according to the raw outline of the shell (black, dashed line). For the determination of the shell roundness the longest (*d*(max)) and shortest (*d*(min)) axis of an ellipse fitted to the outline (yellow, dotted line) were divided by each other. The incidences of the ecophenotypes '*Biorbulina bilobata*' and '*Orbulina suturalis*' were counted manually. **(b)** In *Globigerinoides sacculifer* three morphotypes were distinguished, which are shown here in standard orientation with the apertural plane horizontally oriented and facing the viewer. The position of the twelve landmarks that were extracted for morphometric analyses are indicated as blue dots and exemplarily numbered in the drawing of the trilobus-morphotype (compare Table 1). The upper row shows type specimen drawings of the three morphotypes: trilobus-morphotype from Reuss [51], quadrilobatus- and sacculifer-morphotypes from Banner and Blow [52]. The lower row shows corresponding light microscopy photographs from the studied samples. Scale bars for all light microscopy images equal 100 µm.

Bimodality was tested using Hartigan's dip test [53] as implemented in the R-package 'diptest' v. 0.75-5.

In *O. universa* the extracted morphological parameters were subjected to traditional morphometric analyses (i.e. uni- or multivariate analyses of e.g. length measurements). For *G. sacculifer* we used traditional morphometrics as well as geometric morphometric analytical methods (i.e. multivariate analyses of landmark configurations) as described in Claude [54]

**Table 1.** Description of landmarks used in *Globigerinoides sacculifer* in standard orientation (compare Figure 2) and their associated landmark type after Bookstein [55].

| Landmark | Description | Landmark type |
|---|---|---|
| **1** | Leftmost point of aperture | III |
| **2** | Topmost point of aperture in middle part (point of maximum curvature) | II |
| **3** | Rightmost point of aperture | III |
| **4** | Trisection between aperture, second-youngest, and third-youngest chamber | I |
| **5** | Lowermost point of aperture in middle part | III |
| **6** | Left intersection between youngest chamber and older shell | I |
| **7** | Left point of maximum curvature of youngest chamber | II |
| **8** | Topmost point of youngest chamber | III |
| **9** | Right point of maximum curvature of youngest chamber | II |
| **10** | Right intersection between youngest chamber and older shell | I |
| **11** | Intersection between third-oldest chamber and older shell | I |
| **12** | Intersection between second-oldest and third-oldest chamber | I |

and Zelditch, Swiderski and Sheets [56]. The landmark coordinates were fully Procrustes fitted using the R-package 'shapes' v. 1.1-9 to eliminate size, translation, and rotation as influential factors. The centroid sizes were used as size parameters for *G. sacculifer* shells. To eliminate the influence of size on the traditional morphometric parameters we calculated Mosimann shape vectors [57] as normalized shape descriptors, by normalizing all values per specimen for the geometric mean of all values per specimen.

We further tested all observed morphological trends against three potential models of phyletic evolution using the R-package 'paleoTS' v. 0.4-4 [58]. This allows to distinguish between directional selection (general random walk), a directional pattern due to the accumulation of random change (unbiased random walk), and a system that does not change over time (stasis). The corrected Akaike information criterion ($AIC_c$, [59]) was used to decide, which model best describes the data. Correlations between morphology and other parameters were tested by partial least squares regression (PLSR) as implemented in the R-package 'pls' v. 2.4-3 [60].

Using the Procrustes landmark coordinates we could describe and analyse the shape of *G. sacculifer* specimens. Shape is here defined as the Riemannian shape distance [61] of an individual landmark configuration from the grand mean shape. We calculated the disparity of *G. sacculifer* populations as variance of individual Riemannian shape distances within the population, with confidence intervals and standard errors derived by bootstrapping [56]. Superimposed landmark data were analysed for differences between groups using non-parametric multivariate analysis of variance (NPMANOVA [62]) as implemented in the R-package 'vegan' v. 2.0-10 and canonical variates analysis (CVA [63]) from the R-package 'MASS' v. 7.3-31 [64].

## Results

### Abundance of species and morphotypes

The abundance patterns of *O. universa* and *G. sacculifer* reveal a fundamentally different behaviour of the two species (Figure 3). *Orbulina universa* occurred at generally low abundances that never exceeded 15 % of the total assemblage of planktonic Foraminifera. The species shows two abundance peaks in the studied interval, both followed by a decline in relative and absolute abundance, in case of the second event leading to local extinction. The first abundance peak occurred around 476 ka, and was followed by a rapid decline of both absolute and relative abundance which reached minimum values at 474 ka. After the community was re-established to high abundances at around 473 ka, a second decline was observed. During this second abundance drop the absolute abundances gradually decreased, while the relative abundance remained more constant and showed a much stronger decline at the end. The second abundance decline culminated in the local extinction of the species at 466.5 ka. On the basis of the abundance, we could separate the *O. universa* population into two subsets (indicated as Intervals 1 and 2 respectively in Figure 3) and

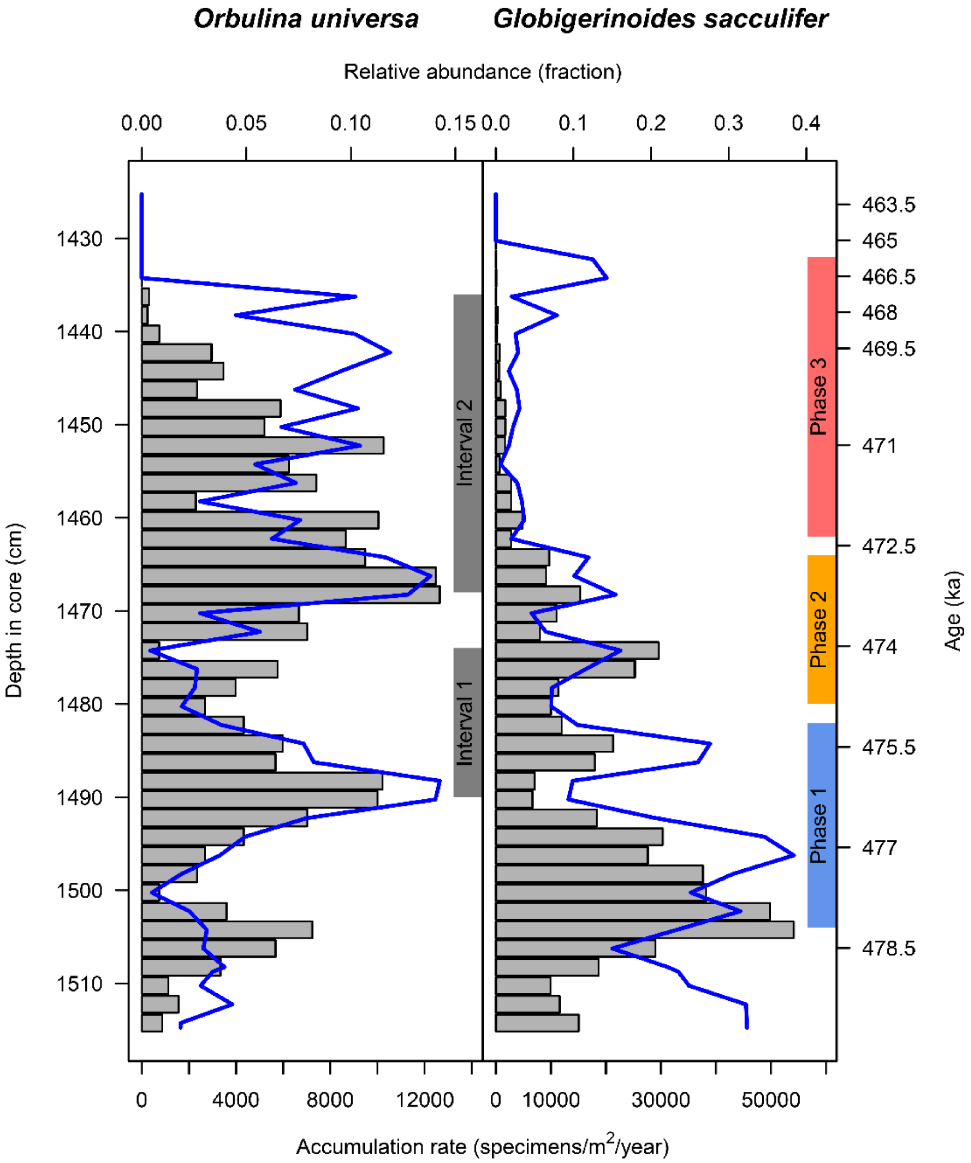



**Figure 3. Abundances of studied species.** Accumulation rates (grey bars) and relative abundances in relation to other species of planktonic Foraminifera (blue lines) of the two species studied here during Marine Isotope Stage 12 in piston core Geo-TÜ KL09 from the Red Sea. Accumulation rates were calculated on the basis of the age model by Rohling, Grant, Bolshaw et al. [25]. The aplanktonic zone begins at approximately 465 ka. The two intervals of dropping abundance for *O. universa* and the three phases defined for *G. sacculifer* on the basis of relative abundances are indicated.

treat them as a replication of the same general process.

*Globigerinoides sacculifer*, on the other hand, showed a gradual reduction in abundance over the course of the entire investigated time interval and generally occurred at higher abundances of up to 40 % of the entire planktonic Foraminifera community. From *c.*478 ka the abundance of the species decreased until a local extinction at 465.7 ka occurred, approximately 1000 yrs later than the comparable event in *O. universa*. The decline in relative abundance is more gradual while the absolute abundance shows stronger fluctuations, but overall both show the same trend. On the basis of the species' relative abundance we defined three phases of population size leading to the extinction (Phases 1–3 in Figure 3). Phase 1 with high abundances (24 %) spans between 478.2 ka and 474.9 ka, Phase 2 with medium abundances (10 %) between 474.9 ka and 472.5 ka, and Phase 3 with low abundances (4 %) between 472.5 ka and 465.7 ka. Those phases could be explicitly investigated for morphological discrepancies in the community. It is further noteworthy that the local extinction of *G. sacculifer* (*c.*465.7 ka) occurred after the local extinction of *O. universa* (*c.*466.5 ka).

In *O. universa* the abundance of two traditionally distinguished morphotypes, viz. '*B. bilobata*' and '*O. suturalis*', have been investigated (Figure 4). Taken together, they occurred with a mean abundance of 2.4 % within the investigated community. '*Biorbulina bilobata*' occurs in marginally higher abundances (on average 1.4 %) than '*O. suturalis*' (on average 0.9 %). The data make the impression as if the abundance of abnormal morphotypes would decrease after approximately 471.6 ka. However, due to their generally low abundance the multinomial confidence intervals on the abundance of the abnormal morphotypes are huge, and when they are taken into account no trend in their abundance can be discovered over the course of the investigated time interval.

When investigating the occurrence of morphotypes in the *G. sacculifer* plexus (Figure 5) we find higher abundances of the sacculifer-morphotype of up to 24 % of the *G. sacculifer* community, broadly coinciding with Phase 1 of the abundance of the species. During early Phase 2 this morphotype decreased rapidly in abundance and, although it never vanished completely within the limits of confidence, never comprised more than 10 % of the population from the second half of Phase 2 onwards. When considering the three Phases separately it is revealed that the abundance of the sacculifer-morphotype differs significantly ($p < .001$) between all three Phases according to a two-proportions *z*-test, with decreasing mean incidence of that morphotype from Phase 1 (14.7 %) over Phase 2 (5.8 %) to Phase 3 (1.9 %). The coiling direction (Figure 5) of the specimens seems to tend a little more to dextral coiling during Phase 1 (59 %) than during Phases 2 (51 %) and 3 (49 %). Accordingly, a two-proportions *z*-test confirms that the incidence of dextrally coiled specimens differs between Phase 1 and Phases 2 ($p = .020$) and 3 ($p < .001$), respectively, but that the abundance of dextral coiling is not statistically different between Phases 2 and 3 ($p = .363$).

## Morphology of *Orbulina universa* during replicated drops in abundance

Morphological parameters of *O. universa* are presented in Figure 4. The shell size of *O. universa* specimens (expressed as Feret diameter) indicates the existence of two phases. The first phase, with comparably large shells, reaches from the beginning of the investigated time interval to approximately 472.1 ka. The shell size than decreased relatively fast between 472.1 and 471.0 ka, and remained relatively stable afterwards. The distribution of sizes within samples reveals that the decrease in mean size of the population was mainly caused by an increase in the abundance of small specimens. Large specimens became only marginally rarer, being more common before, but never disappeared from the sedimentary record. The upper part of the profile (starting at approximately 472 ka) makes the impression as if the size distribution of shells of *O. universa* could be bimodal. Indeed, this bimodality is significant in six and only marginally insignificant in a further two samples within the part of the profile younger than 472.4 ka (File S1). The small increase in size variation contemporaneous with the decrease in shell size is probably a result of this developing bimodality in the community.

To investigate shell roundness in *O. universa* we excluded '*B. bilobata*' and '*O. suturalis*' specimens (59 specimens) from the analyses because both deviate significantly from the normal morphology of a terminal shell of that species. The thus derived shell roundness of *O. universa* also shows two phases during the investigated time interval. Shells in the beginning of the section are rather round. At around 472.1 ka shells began to become less round rather rapidly and the community reached a second steady stage of reduced shell roundness approximately 500 yrs later. This reduction in shell roundness thus started contemporaneously with the decrease in shell size, but the second stage of a stable reduced shell roundness was reached faster than the second stage of shell size. Additionally, in contrast to shell size, the shell roundness shows an increase in variation (which is significant on average judged by the

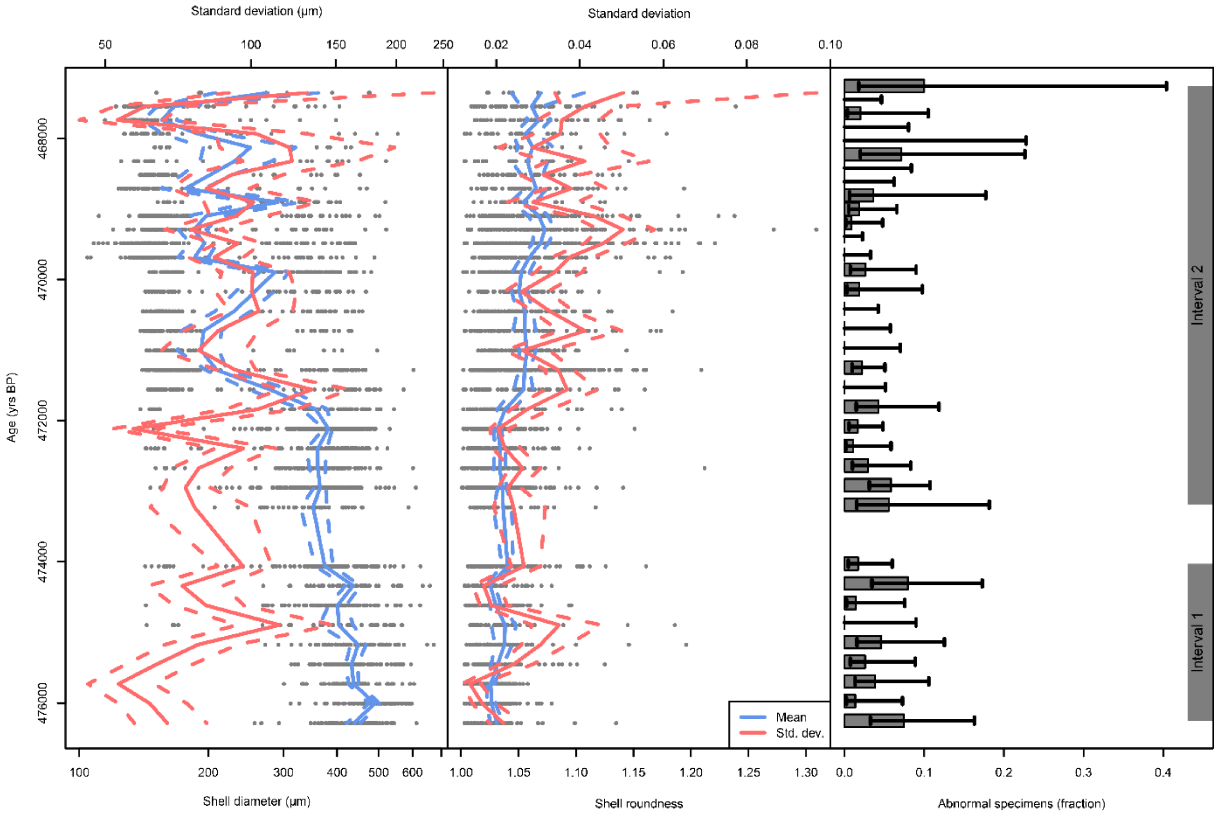



**Figure 4. Morphology of *Orbulina universa*.** Morphological parameters of specimens of *O. universa* from Marine Isotope Stage 12 in piston core Geo-TÜ KL09 from the Red Sea. Shell size is expressed as Feret diameter, shell roundness as ratio between long and short axis of an ellipse fitted to the shells silhouette (a value of one would represent a sphere). For both parameters, raw values (grey dots) are plotted alongside the sample mean and standard deviation (solid lines) together with their 95 % confidence intervals (dashed lines). The incidence of abnormal specimens is plotted as grey bars together with their multinomial 95 % confidence intervals as error bars. The two intervals of decreasing abundance (compare Figure 3) are indicated on the right side of the plot. Note the log-scaling of the *x*-axis for the shell diameter.

confidence intervals of the standard deviation) contemporaneously with the decrease in shell roundness (Figure 6a).

## Morphology of *Globigerinoides sacculifer* during a long, continuous extinction event

The shell size (expressed as centroid size, Figure 5) in *G. sacculifer* shows a comparable pattern to the incidence of the sacculifer-morphotype, with larger shells during Phase 1, a rapid size decrease during Phase 2, and small shells during Phase 3. Comparing the values within the phases reveals a decrease in mean shell size from Phase 1 (408 µm) over Phase 2 (288 µm) to Phase 3 (238 µm). The differences in size are significant between all groups ($p < .001$ for a Kruskal–Wallis test, with all $p < .001$ in pairwise Mann–Whitney *U* tests). Moreover, judged by the 95 %-confidence intervals, the variation of shell size decreased significantly at the end of Phase 2 (Figure 6b). It is obvious that this decrease in variation, as well as the general shell size decrease, is nearly exclusively caused by the lack of large specimens after Phase 2, while the size of the smallest specimens remained rather constant during the entire interval investigated.

To eliminate the size-effect in certain parameters during a traditional morphometric analysis, we calculated Mosimann shape vectors for the parameters shell size (as centroid size of the whole landmark configuration), aperture size (as centroid size of landmarks 1–5), and size of the terminal chamber (as centroid size of landmarks 6–10), which are presented in Figure 7. Statistics for the results presented hereafter are also summarized in Table 2. The normalized shell size reveals an increase in shell size between all three phases, which is supported by a Kruskal–Wallis test between all three Phases ($p < .001$). Additionally, after a significant Kruskal–Wallis test ($p < .001$) a pairwise comparison shows that the normalized aper-

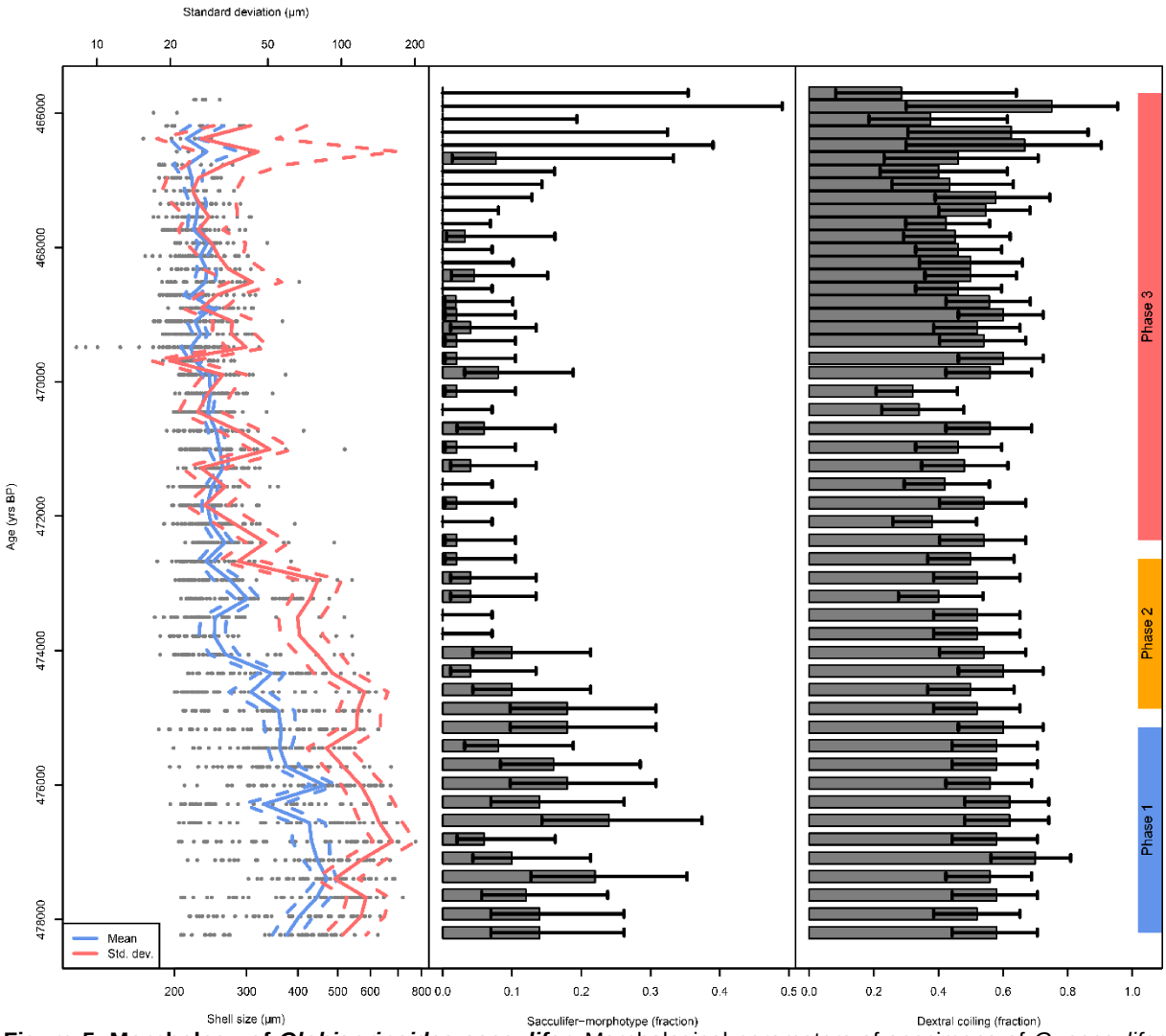



**Figure 5. Morphology of *Globigerinoides sacculifer*.** Morphological parameters of specimens of *G. sacculifer* from Marine Isotope Stage 12 in piston core Geo-TÜ KL09 from the Red Sea. Shell size is expressed as centroid size of landmark data (compare Figure 2), and plotted are the raw individual values (grey dots) alongside the sample mean and standard deviation (solid lines) and their 95 % confidence interval (dashed lines). The proportion of the sacculifer-morphotype and dextrally coiled specimens in the *G. sacculifer* community are shown as grey bars on which the multinomial 95 % confidence intervals are indicated as error bars. The three phases defined by abundance (compare Figure 3) are indicated on the right side of the plot. Note the log-scaling of the *x*-axis for the shell size.

ture size was likely constant during Phases 1 and 2, but dropped during Phase 3. However, it seems that the majority of this significant drop in aperture size can be attributed to the first *c.*4500 yrs of Phase 3, while during the last *c.*2000 yrs of Phase 3 the aperture size seemingly started to increase again. A similar pattern is revealed for the normalized size of the terminal chamber (Kruskal–Wallis test, $p < .001$), which significantly increased in Phase 3 after it had been stable during Phases 1 and 2. Another interesting development is revealed by the variation of the Mosimann shape vectors. All three parameters seem to indicate a drop in variation during the early stages of Phase 3, coinciding with the time interval during which aperture size has been lowest. When considering the confidence intervals on the standard deviations, this pattern seems to vanish in terminal chamber size and is debatable in aperture size, but it remains in shell size. Again more or less coinciding with the renewed increase in aperture size during the last *c.*2000 yrs of Phase 3 we observe a significant increase in the variation of all three parameters (compare Figure 6b). While this can partly be attributed to inflating confidence intervals as a result of small sample size during that time, the earlier part of that termi-

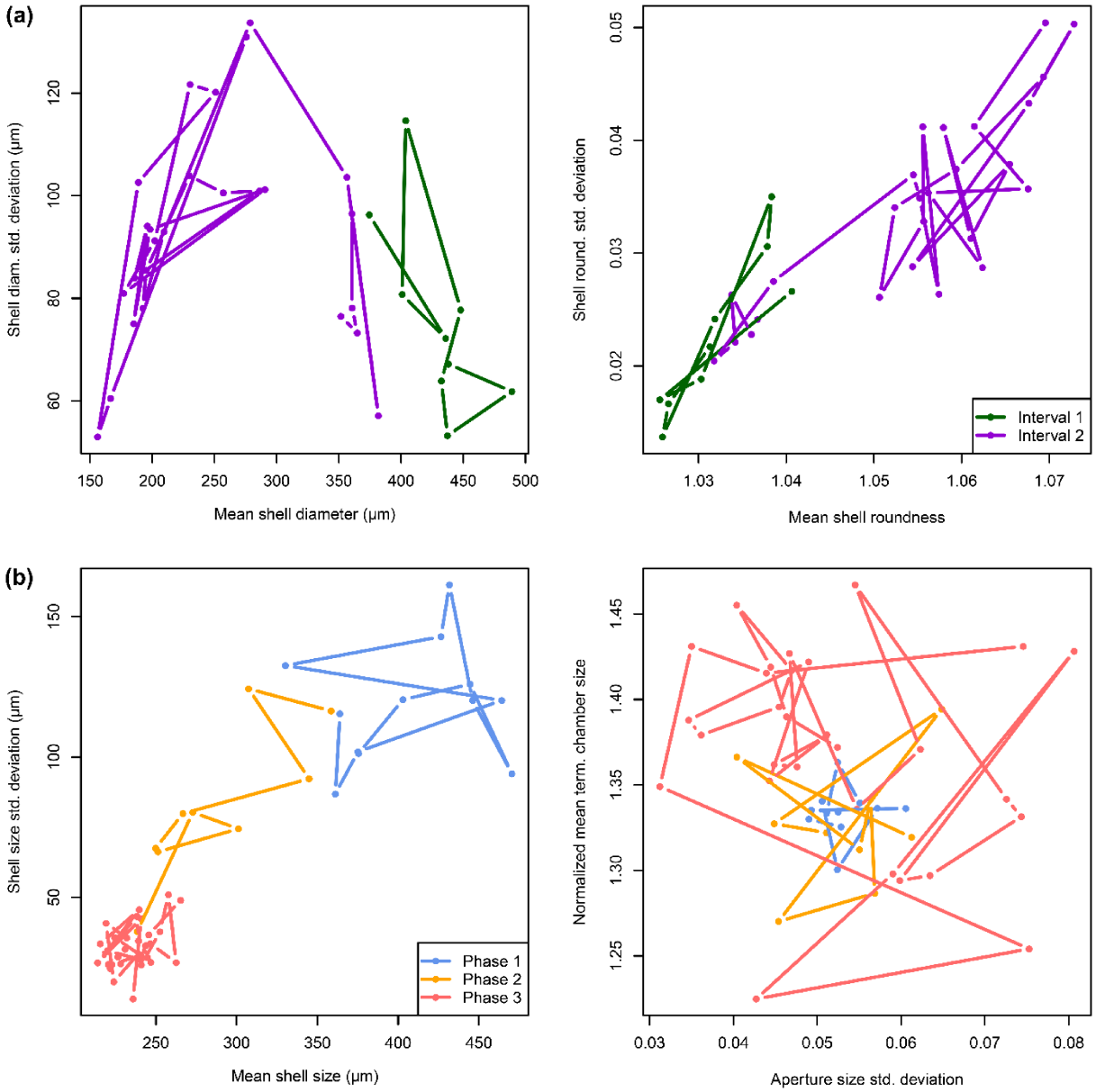



**Figure 6. Crossplots of morphological parameters.** Mean values per sample (points) are connected with lines in temporal order. **(a)** Parameters for *Orbulina universa*. The population-wide variance of shell diameter is rather constant, but due to the increase in smaller and decrease in larger specimens the mean shell diameter declines over time. In contrast, the shell roundness shows increasing deviation from sphericity and increasing variation towards the local extinction. **(b)** Parameters for *Globigerinoides sacculifer*. Both the mean shell size and it's variance decrease dramatically when the species approaches its local extinction. The normalized size of the terminal chamber is on average largest during the later part of the profile, when the variation of the aperture size shows highest values as well.

nal stage still has large enough sample sizes to indicate that this trend truly exists, and probably remains until local extinction in the aplanktonic zone, but cannot be reliably identified during the latest Phase 3.

Using geometric morphometrics allows an even more detailed analysis of the shape of *G. sacculifer* specimens as a whole (Figure 8a). With such data, morphology can be efficiently presented, either using the superimposed landmarks themselves or the Riemannian shape distance of each individual from the grand mean shape of all investigated specimens after Procrustes fitting. In general agreement with indications by traditional morphometrics, the shape of *G. sacculifer* specimens between Phases differs significantly (NPMANOVA using Euclidean distance measure on landmark data, 999 permutations, $p < .001$). A pairwise test of shape differences using the 'testmeanshapes()' function of the R-package 'shapes' (999 permutations) reveals, that the shape of *G. sacculifer* specimens significantly

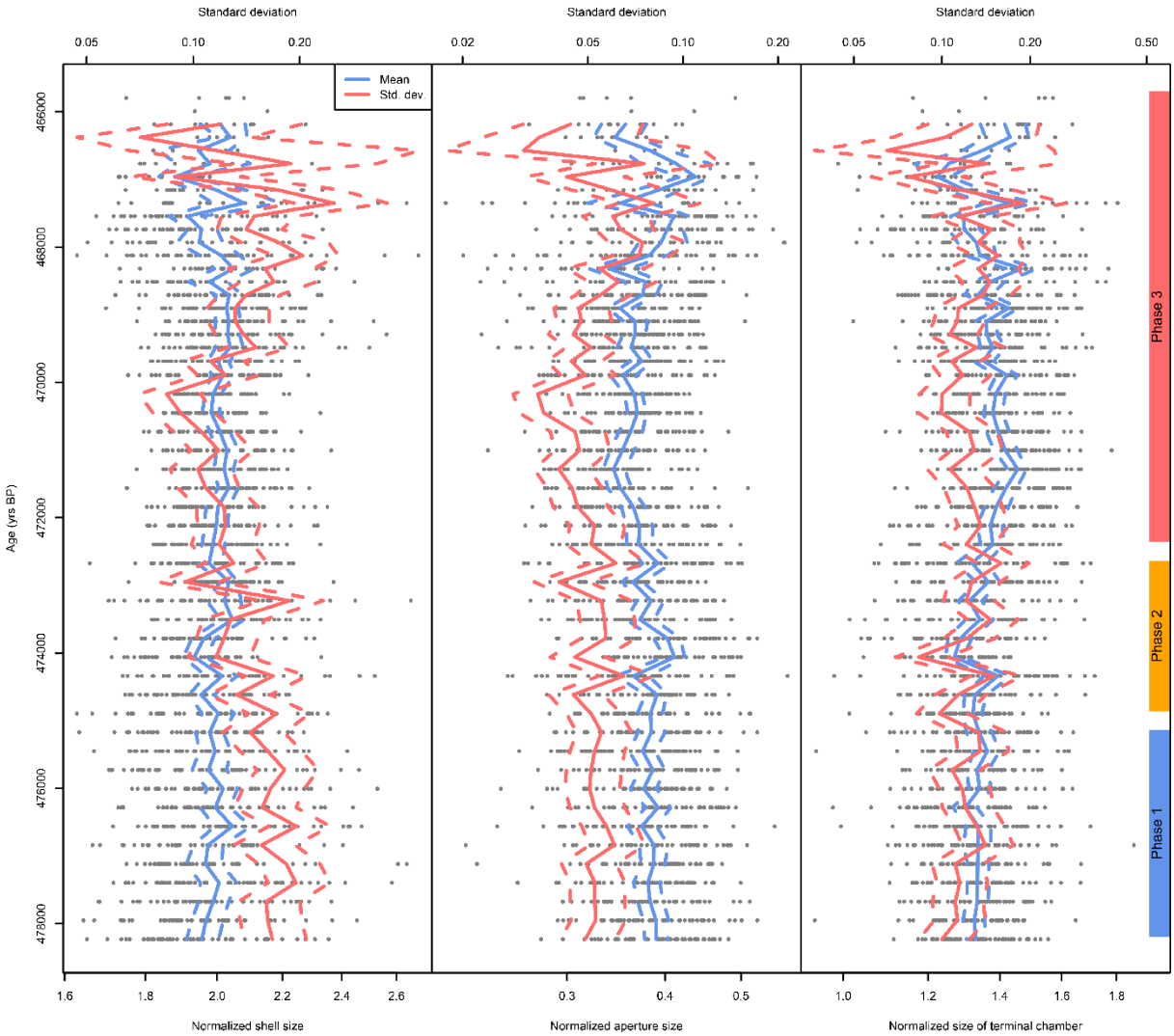



**Figure 7. Mosimann shape vectors of *Globigerinoides sacculifer*.** Mosimann shape vectors on the basis of the geometric mean of specimens of *G. sacculifer* from Marine Isotope Stage 12 in piston core Geo-TÜ KL09 from the Red Sea. Individual values for normalized shell size, aperture size, and size of the terminal chamber (grey dots) are plotted alongside their sample mean and standard deviation (solid lines) with their respective 95 % confidence intervals (dashed lines). The three phases defined by abundance (compare Figure 3) are indicated on the right side of the plot. Note the log-scaling of the *x*-axes.

differs between all three phases at $p < .001$. The shape here reveals the same pattern as already observed in some Mosimann shape vectors, i.e. the shape is especially different during the first *c.*4500 yrs and switches back to an earlier stage for the last *c.*2000 yrs of Phase 3 (Figure 8a). As with the Mosimann shape vectors, during this terminal stage the shape seems to become more variable, but this may well be a coincidence of the sample sizes plummeting at that point. When analysing the shape change from phase to phase, as depicted in Figure 9, it is revealed that from Phase 1 to Phase 2 the terminal chamber became flatter (but without becoming broader) and the aperture became more spherical, mainly by increasing in height. At the same time the lower part of the shell containing the older chambers became more voluminous, so that there is a general trend towards a smaller terminal chamber in relation to the older chambers. This trend is reversed in Phase 3, but not by returning to the shape present during Phase 1. Rather, the terminal chamber increased in size into all directions (becoming more inflated), while the older part of the shell remained in the state it had during Phase 2. This led to a strong increase in the size of the terminal chamber in regard to the older shell, as already observed with the Mosimann shape vectors.

A reliable way to describe phenotypic plasticity in geometric morphometrics is the disparity of a community. A higher disparity

**Table 2.** Comparison of Mosimann shape vectors for shell size, aperture size, and size of the terminal chamber and disparity as inferred from Riemannian shape distances in Procrustes superimposed landmark configurations in *G. sacculifer* specimens from core Geo-TÜ KL09 during Marine Isotope Stage 12. The table shows results of pairwise Mann–Whitney *U* tests for Mosimann shape vectors and a *t*-test (with adjusted degrees of freedom) for disparity, with *p*-values in each case adjusted for the false discovery rate. Phase durations are depicted in Figure 3.

| Phase pair | | Shell size | | Aperture size | | Terminal chamber size | | Disparity | |
|---|---|---|---|---|---|---|---|---|---|
| | | *U* | *p* | *U* | *p* | *U* | *p* | *t* | *p* |
| **Phase 1** | **Phase 2** | 128268 | .166 | 133961 | .831 | 139691 | .335 | 2.735 | .003 |
| **Phase 1** | **Phase 3** | 299764 | .001 | 389977 | < .001 | 268393 | < .001 | 8.868 | < .001 |
| **Phase 2** | **Phase 3** | 236678 | .141 | 293443 | <. 001 | 195541 | < .001 | 5.519 | < .001 |

indicates higher phenotypic plasticity within the population in comparison to a population with lower disparity. Differences in disparity between groups can be validated using Student's *t*-test that has been modified for the number of degrees of freedom (number of specimens in both groups minus two). Applying those analysis to our data reveals that disparity is highest in Phase 1, mediocre in Phase 2, and lowest in Phase 3, with differences between all phases being significant (Figure 8b, Table 2). This implies a continuously decreasing phenotypic plasticity of the community from Phase 1 to Phase 3.

All results have been sincerely tested against potential sources of error and resulting misinterpretations, and no related problem was discovered (File S1, Section 2).

## Discussion

### Stabilisation and disruption within populations of planktonic Foraminifera

The concept of phenotypic plasticity [15] is here used as a neutral term for the observed morphological variation among specimens of a population, and is thus contrasted to variability, which is the potential to vary within the borders of the genetic encoding [65]. This is especially important in planktonic Foraminifera, where the existence of (pseudo-)cryptic species can lead to an underestimation of genetic diversity and thus variability of the population. In *G. sacculifer* we can rule out that possibility, because despite its large morphospace it only contains one biological species with high phenotypic plasticity [39]. In *O. universa*, on the other hand, the morphospecies encompasses several biospecies [66]. At present only one genotype is known to occur in the Red Sea [67], but the multimodality in shell size of *O. universa* we observe in the upper part of the profile points towards the existence of more than one sub-population. While at the moment we do not know of any instance were pseudo-cryptic species in *O. universa* can be differentiated by shell size [68], we must be especially careful in the interpretation of results obtained from that species.

Importantly, by controlling for cryptic speciation we could assume that the populations investigated in our study share a comparable genetic code per species, and therefore the variability of the populations should not change dramatically over the time of the natural experiment. This in turn would imply, that the morphological changes we observe in the studied interval are results of a changing variation of a homogeneous community, and therefore the effect of environmental forcing associated with the onset of the aplanktonic zone.

We see a generally different development in shape in the two species investigated. In *O. universa* we observe a decrease in size and mean roundness during the later part of the profile (Figure 4, Figure 6a), when the abundance dropped and the species approached its local extinction in the aplanktonic zone (Figure 3). Both trends conform best with the assumption of an unbiased random walk pattern, but a general random walk pattern is equally possible ($\Delta AIC_c = 1.82$ for shell size and $\Delta AIC_c = 1.31$ for shell roundness) [69]. This implies that the observed morphological change could be the result of accumulation of random changes, but could also be the imprint of directional selection. In any case, the observed trends are significant, and the model does not comply with the assumption of stasis ($\Delta AIC_c = 50.75$

for shell size and $\Delta AIC_c$ = 42.21 for shell roundness).

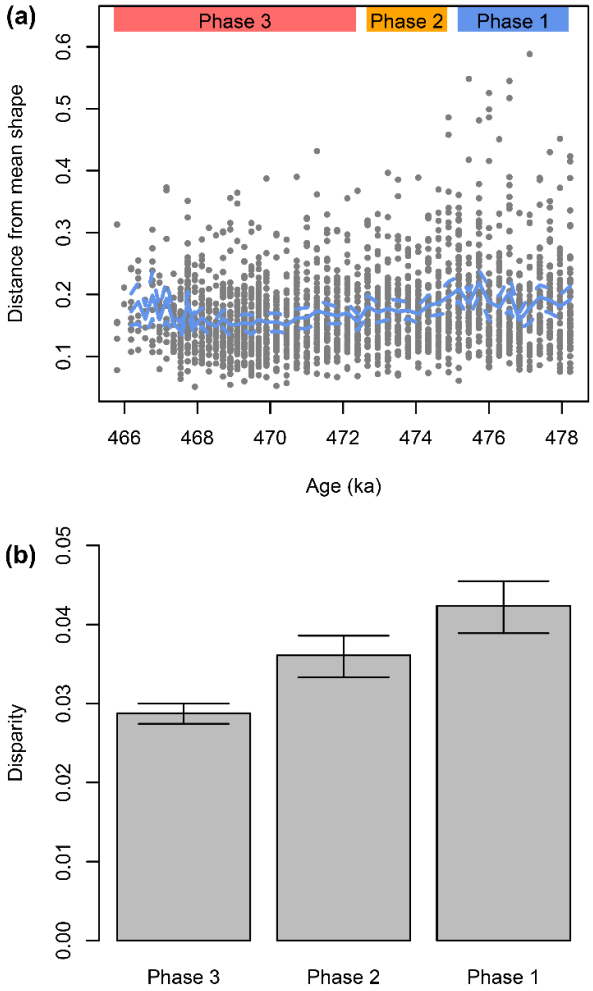



**Figure 8. Shape and disparity of *Globigerinoides sacculifer*. (a)** Shape of *G. sacculifer* specimens from Marine Isotope Stage 12 in piston core Geo-TÜ KL09 from the Red Sea, expressed as Riemannian shape distance from the grand mean. Individual values (grey dots) are plotted alongside sample means (solid line) and their bootstrapped 95 % confidence interval (dashed lines). **(b)** Disparity (expressed as variance of the Riemannian shape distance from the grand mean) of the *G. sacculifer* community within the three phases of decreasing abundance indicated in subfigure (a) (compare Figure 3), together with bootstrapped 95 % confidence intervals (error bars).

The shell size shows bimodality, and while the shell roundness seems to remain unimodally distributed within the population, it shows a significant increase in variance. Both trends indicate disruptive selection and decanalization in the *O. universa* population in the upper part of the profile, coinciding with increasing salinity as a result of sea level decrease (Figure 10). It is important to note that disruptive selection can, but does not necessarily have to, lead to a bimodality in the trait distribution. Especially under random mating models disruptive selection can introduce a broadening of the reaction norm, without inducing bimodality [70], and the degree of disruption can vary in different traits, as is supported by the theory of morphological modularity [65, 71, 72]. Given that we thus see an increase in morphological plasticity in both size and roundness of shells of *O. universa* it is important to understand the implication of that pattern, given that we cannot fully constrain genetic diversity in this species. The observed pattern would be consistent with two possible end-member scenarios: (1) a monospecific *O. universa* population present at the time in the Red Sea shows signs of disruptive selection as a result of exposure to a suboptimal environment [14], or (2) a new population comprising a different biospecies with different morphology is introduced into the Red Sea.

To investigate those scenarios we applied a Spearman rank-order correlation between individual shell size and shell roundness and found that they are significantly correlated ($\rho = -0.424$, $p < .001$, Figure 11a). Having thus reason to believe that smaller individuals also tend to have less round shells we artificially divided the population into two subsets at the approximate local minimum of the size distribution (225 µm) and tested the shell roundness in both subgroups against each other with a Mann–Whitney *U* test. The test implies that the subpopulation with larger shells also produced shells that are on average significantly ($p < .001$) more round (mean = 1.04) than the smaller subpopulation (mean = 1.07). The fact that the minimum roundness value (i.e. highest shell roundness) is practically equal (1.00) in both subpopulations shows, that the observed trend is also not the result of a lower precision of shell roundness determination in smaller shells. On the other hand, comparing the coefficient of variation shows a significantly higher variation of shell roundness in the group with smaller shells (0.038) than in the group with larger shells (0.021) (Figure 11a), which cannot fully be the result of the trends observed (Figure 4) because the group with

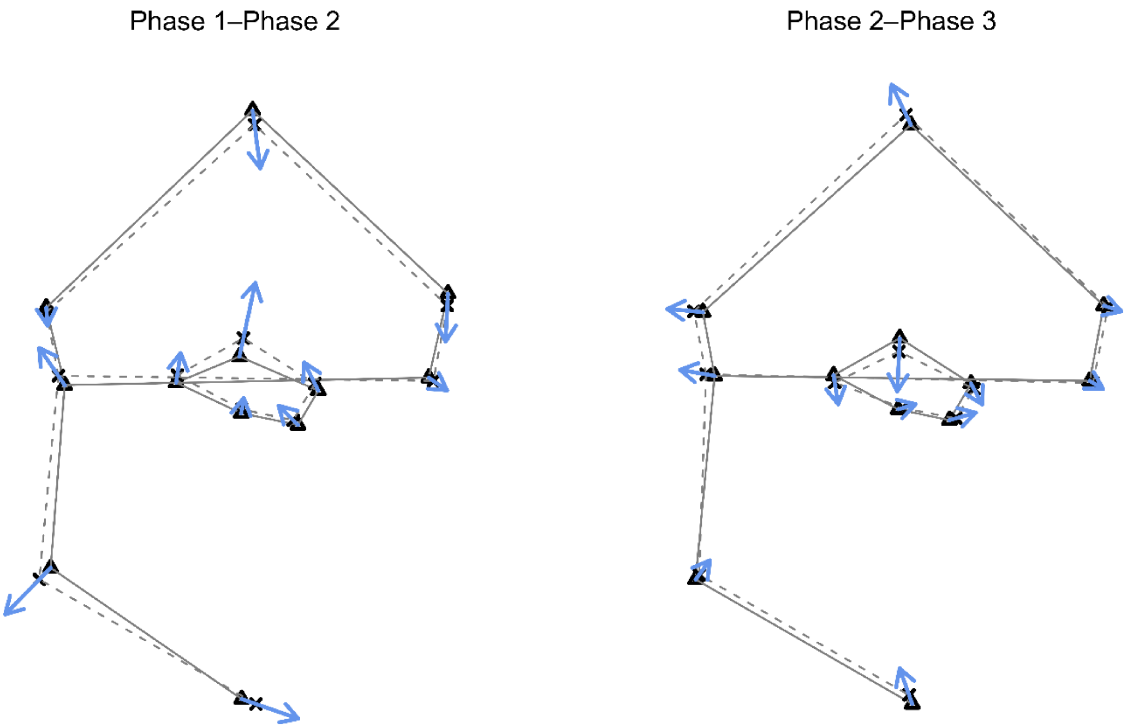



**Figure 9. Shape change in *Globigerinoides sacculifer*.** Shape change of *G. sacculifer* specimens from Marine Isotope Stage 12 in piston core Geo-TÜ KL09 from the Red Sea between the three abundance phases (compare Figure 3). Triangles and solid lines depict the initial state, crosses and dashed lines show the derived state, arrows show the direction of shape change (three times amplified). From Phase 1 to Phase 2 the terminal chamber becomes flatter (landmarks 8, 7, and 9), the aperture more round (landmark 2), and the lower part of the shell becomes more voluminous (landmarks 11 and 12). From Phase 2 to Phase 3 the terminal chamber becomes inflated (landmarks 6–10) and the aperture becomes slightly flatter again (landmark 2) but not as flat as during Phase 1.

small specimens also contains some specimens from the early part of the profile and the group with large specimens contains several specimens from the later part of the profile. This is evidenced when shell roundness of the small group is plotted against time, which shows that all roundness values prevailed at all times and there is no temporal trend in the data (Figure 11b).

Taken together those observations have some serious implications on the interpretation of the observed morphological trends in *O. universa* shells. They imply that the Red Sea was possibly invaded by a population with smaller, less spherical final chambers from approximately 473 ka onwards, which increasingly established its presence at the expense of the incumbent. Should this be true, we would find here the first example where different *O. universa* biospecies could be distinguished on the basis of relatively easily obtainable morphological characters [68] and also the first example of more than one *O. universa* biospecies occurring in the Red Sea [67]. Furthermore, it would provide evidence for different ecological preferences among those biospecies, which facilitates competitive exclusion due to increasing stress levels. Against this scenario, however, would speak that the potentially invading species would have had to be of Indian Ocean origin. Within an environmental setting of increasing salinity, it is hard to perceive that any species from the open-marine Indian Ocean would have a selective advantage over a native species from the Red Sea, that should be better adapted to high salinities [73].

Alternatively, it is also possible that shell roundness and shell size are biologically integrated in *O. universa*, so that a change in one parameter necessitates a certain change in the other value [72]. This would be an alternative explanation for the observed correlation between individual shell size and shell roundness, but the very low covariance between both parameters (−0.007) together with the fact that small specimens realize the complete range of possible deviations from sphericity principally argues against that

hypothesis. Should the investigated *O. universa* population nevertheless comprise only one biospecies which shows signs of disruptive selection, this would replicate results obtained on a Mediterranean population that was exposed to higher stress levels in relationship with the onset of Sapropel deposition [22]. Such an observation would lend valuable evidence to the assumption, that certain morphological changes can be universally interpreted as indicator of environmental stress, at least as long as the same species is considered.

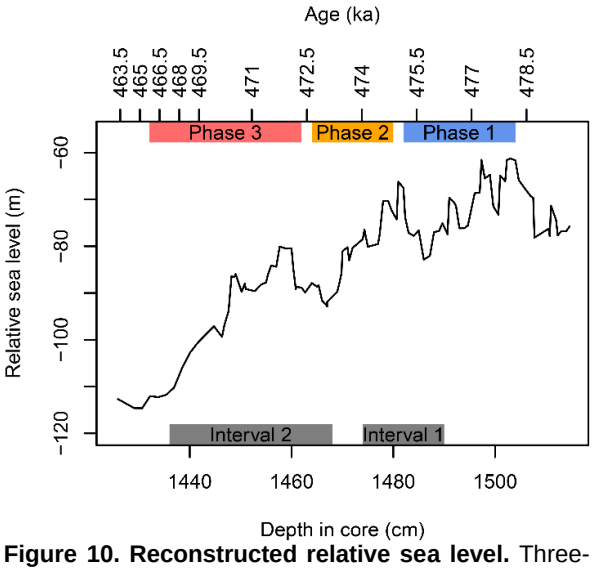



**Figure 10. Reconstructed relative sea level.** Three-point moving average relative sea levels in the Red Sea during Marine Isotope Stage 12 in core Geo-TÜ KL09, as calculated by Rohling, Grant, Bolshaw et al. [25] on the basis of $\delta^{18}O$ values of *Globigerinoides ruber*. Since salinity in the Red Sea is mainly driven by sea level [32], this curve can be considered to be the inverse of sea surface salinity at first approximation. The intervals (for *Orbulina universa*) and phases (for *Globigerinoides sacculifer*) defined by abundance (compare Figure 3) are indicated in the plot.

Mechanistically it has been hypothesized that shell size in *O. universa* is correlated with either water salinity, where larger shells are produced in less saline water to counteract buoyancy [74], or nutrient availability, where larger shells are produced under eutrophic conditions because the surplus of food provides the energy needed for the construction of such a larger shell [75]. Our observations could be in line with the salinity hypothesis, since the sea level decrease caused a salinity increase [32] and would thus have been favoured the production of smaller shells. On the other hand, a reduction in shell size has also been proposed to indicate suboptimal environmental conditions [76, 77]. Since the strong salinity increase in the Red Sea associated with the onset of the aplanktonic zone increased the water salinity (which is generally high in the Red Sea, compare Sofianos and Johns [73]) to even higher levels, necessarily shifting the local habitat away from the optimum requirements of the species, it is therefore hard to say whether the trends we observe are the result of salinity changes (as purely abiotic forcing), increased environmental stress (as biotic forcing) or a mixture of both. However, if the observed morphology change would be a purely biotic stress response, assuming that abundance is a useful proxy for the suitability of the environment and thus environmental stress, we would principally expect to see a comparable development in Interval 1, which ends with a strong abundance drop, as in Interval 2, which ends with the local extinction of the species. In contrast to that assumption, neither shell size nor shell roundness show any signs of a deviation associated with that first abundance drop, which is also supported by the fact that both shell size and shell roundness are significantly different between both intervals at $p < .001$, which is not what one would expect if they would show a comparable pattern.

*Globigerinoides sacculifer*, on the other hand, draws a completely different picture. While we also see a size-decrease in shells of that species from Phase 2 to Phase 3 (corresponding to a similar signal in *O. universa* but starting already 2000–2500 yrs earlier in *G. sacculifer*), this size decrease is not associated with an increase but rather a decrease in shell size variation (Figures 5, 6b). The same pattern of decreasing variance can be observed in the Mosimann-normalized shell and aperture size (Figure 7), and the shell shape, as indicated by the disparity (Figure 8). In shell size this decrease complies with both the unbiased random walk model and the general random walk model ($\Delta AIC_c$ = 1.78) but the community does not show stasis ($\Delta AIC_c$ = 80.88). The shape of specimens, expressed as distance to the mean shape,

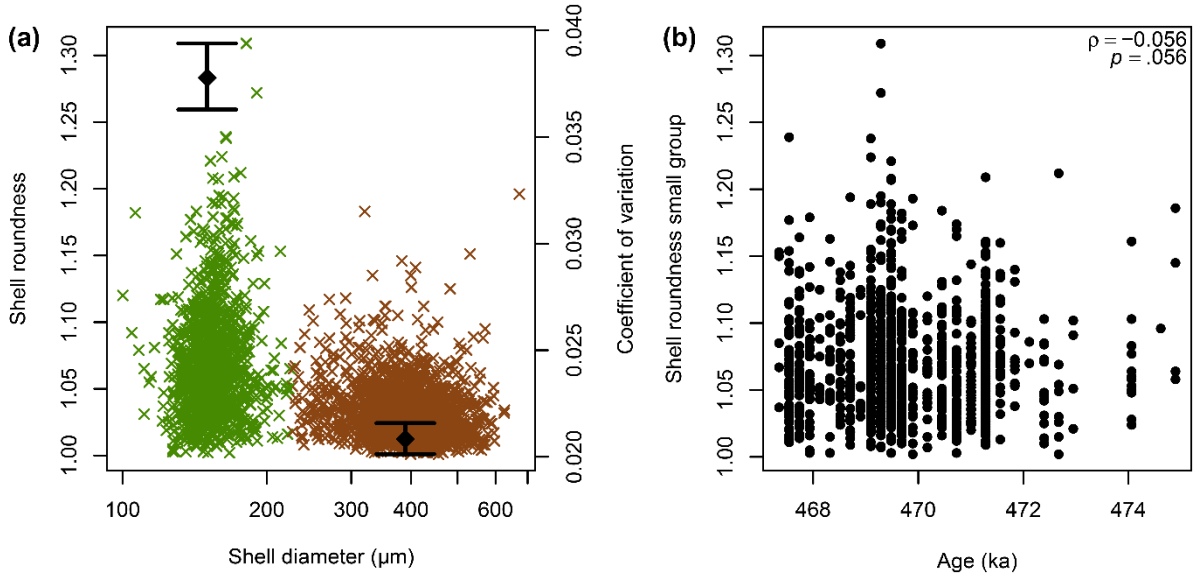



**Figure 11. Correlation between shell size and shell roundness in *Orbulina universa*.** Shell size (expressed as Feret diameter) and shell roundness (as deviation from a circle, which would have a value of one) of *O. universa* specimens in the Red Sea during Marine Isotope Stage 12 in core Geo-TÜ KL09. **(a)** The population has been artificially divided into two subgroups, specimens < 225 µm (green) and specimens > 225 µm (brown), at the local minimum of the size distribution histogram. Within both groups the coefficient of variation (standard deviation divided by mean; black diamonds) and its associated 95 % confidence interval (whiskers, after Vangel [78]) has been calculated. Smaller specimens show a significantly higher variance of roundness than do larger specimens. Note the log-scaling of the *x*-axis. **(b)** This higher variance in the small group cannot be explained by increase of less round individuals toward the end of the profile. A correlation of shell roundness and age exclusively in the small group reveals that all roundness-values were present at all times, and no significant correlation between shell roundness and sample age exists (Spearman rank-order correlation).

clearly supports the stasis model with unbiased random walk ($\Delta AIC_c$ = 2.23) and general random walk ($\Delta AIC_c$ = 4.29) being inferior.

While the size decrease may itself be a signal for decreasing environmental suitability for the species [76, 77], the decrease in variance on several levels is a clear signal for stabilizing selection inducing microenvironmental canalization [15, 79]. It is interesting, that stabilization occurs towards Phase 3, which must have been clearly environmentally suboptimal for *G. sacculifer* (drop in abundance, increase in salinity). This could indicate either a stabilization of the regional environment allowing selection for an optimal trait [13], or a rapidly changing environment enforcing fluctuating selection that benefits a stable phenotype in the long run [80, 81]. Another parameter, which could actually help in solving this problem, is the incidence of dextrally coiled specimens. *Globigerinoides sacculifer* is one of the few species of planktonic Foraminifera which are believed to show directional asymmetry (i.e. one direction is favoured over the other, compare Van Valen [82]) concerning their coiling direction [83]. Yet, according to a *G*-test, in our samples we see signs for such a directional asymmetry only in Phase 1 ($p < .001$), while from Phase 2 onwards the population exhibits signs of antisymmetry (i.e. both coiling directions are equally likely, compare Timofeéf-Ressovsky [84]) ($p = .956$ for both phases). Earlier studies have shown that antisymmetry can be introduced spontaneously into a population that normally exhibits other symmetry patterns when the population is exposed to a rapidly changing environment [85, 86], so that we have good reason to assume that the canalization in *G. sacculifer* in our samples results from fluctuating selection.

The reduction in the abundance of the sacculifer-morphotype after Phase 1 could be another manifestation of the stabilizing selection, but it could also result from an inability of *G. sacculifer* shells to build an asymmetric chamber below a certain chamber size threshold. In the latter case the drop in the abundance of the sacculifer-morphotype would result from the shell size decrease and not necessarily be a signal for stabilizing selection. Since the correlation between the

abundance of the sacculifer-morphotype and both shell size ($\rho$ = 0.735, $p$ < .001) and standard deviation of shell size ($\rho$ = 0.697, $p$ < .001) is nearly equally strong, it is not possible to say, what is actually the case.

The observed morphological trends in *G. sacculifer* over time, as shown in Figure 12, can be interpreted as adaptive for a changing environment that gradually becomes less suitable for the population. During Phase 1 the sacculifer-morphotype was abundant. This phenotype is often assumed to be more abundant under lower stress levels but has already earlier been shown to be correlated with larger shell sizes [87]. During Phase 2, coinciding with a first strong drop in sea level and thus salinity increase (Figure 10), the abundance of the sacculifer-morphotype and the shell size decreased, while the size of the terminal chamber decreased as well, indicating a trend towards Kummerforms that are often associated with unfavourable environmental conditions. Finally, in Phase 3 the shell size decreased further while the terminal chamber became larger again in relative terms. The latter trend could be necessitated by the small shell size, so that the terminal chamber had to become relatively larger again to provide enough space for gametogenesis. This phase is also characterized by a canalization peak, probably induced by an environment that was so unstable and unfavourable for the *G. sacculifer* population that any deviation from a very narrow morphotype would drastically decrease survival rates and fitness.

## Planktonic Foraminifera morphology as environmental proxy

Morphology has often been shown to be influenced by the environment [6, 12, 22] but recently this hypothesis has been challenged. For example, it has been theorized that morphological change may be under circumstances largely decoupled from the environment, because the observed changes in morphology have no impact on fitness [20, 21] and morphology can therefore not serve as basis for selection. Alternatively, it was hypothesized that several developmental traits are buffered by the same genes and are therefore biologically integrated [72, 88-90]. This would imply that an observed change in any morphological trait could be the by-product of a change in another (superficially unrelated) parameter that is the actual basis for selection.

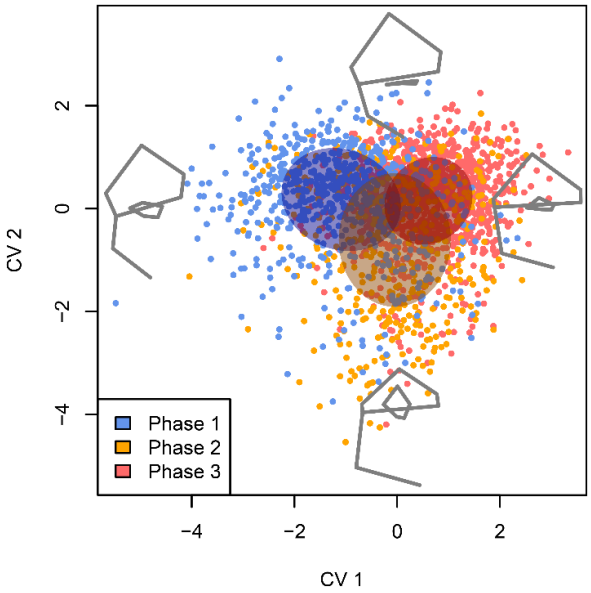



**Figure 12. Canonical variates analysis (CVA) of the shape of *Globigerinoides sacculifer*.** Shape change of *G. sacculifer* specimens from Marine Isotope Stage 12 in piston core Geo-TÜ KL09 from the Red Sea, shown as a CVA scatterplot. Points indicate specimens in the three pre-defined abundance phases (compare Figure 3), ellipses of the same colour indicate the 95 % confidence interval of the standard deviation on the centroid of the respective group, grey silhouettes depict the morphology at the extremal points of the canonical variate (CV) 1 and 2 axes, respectively. The CVA is significant ($T^2$ = 0.620, $F_{approx}$ = 33.171, $p$ < .001) with 84.2 % of the variance explained by CV 1 and the remaining 15.8 % by CV 2, and a correct classification rate of the discriminant function of 68.50 %. While there is a large overlap between groups they also clearly occupy different parts of the morphospace: In Phase 1 (low values on CV 1, around zero on CV 2) specimens have relatively equally sized upper (terminal chamber) and lower (older chambers) shell portions, a tendency for oval to slit-shaped apertures and an asymmetric terminal chamber (sacculifer-morphotype). During Phase 2 (around zero on CV 1, low values on CV 2) the lower shell part increases in size in expense of the terminal chamber, the aperture becomes more round, and the terminal chamber more symmetric. In Phase 3 (high values on CV 1 around zero on CV 2) the aperture becomes more oval again, while the terminal chamber becomes more inflated but remains symmetrical.

We therefore tested the correlation between environment (sea level) and fitness (relative abundance, accumulation rate), and morphology using PLSR. In contrast to other ordination methods, this method is robust against collinearity, which must be assumed

to exist between individual Procrustes fitted landmark coordinates and also between different abundance measures and probably abundance and sea level. In both species we find the PLSR to be significant at $p < .001$. After removing the accumulation rate as additional parameter because it has nearly the same loadings as the relative abundance, the PLSR remains significant at $p < .001$ (with a significant correlation between T and U scores on the first partial axis, $p < .001$). The loading of the relative abundance is dominating the first partial axis, while the sea level mainly explains residual variation on the second axis, but this could be the result of the high collinearity between both variables ($\rho = 0.593$, $p < .001$), which is also evidenced by the fact that a PLSR using only the relative sea level remains significant. While the PLSR thus confirms a relationship between morphology and sea level as well as abundance, it cannot help to disentangle the influence of both parameters in this case. This is also supported by a correlation between shell size and both relative abundance of the respective species and relative sea level. The correlation is significant for both parameters in both species.

Qualitatively, it is obvious that the abundance decrease in *G. sacculifer* begins much earlier (around 477 ka) than in *O. universa* (approximately 473 ka). However, while the abundance decreases steadily in *G. sacculifer* it is more variable in *O. universa* with one abundance lowpoint before the study period and another one at the end of Interval 1. This indicates that abundance in *O. universa* might not be strictly coupled to salinity but also influenced by other factors such as competition with other species. In *G. sacculifer*, however, abundance is likely to mirror salinity changes. Furthermore, *G. sacculifer* becomes extinct 1000 yrs later than *O. universa*, but shows first morphological reactions already during early Phase 2, i.e. *c.*2000 yrs earlier than the deviations in shell size and roundness in *O. universa* occur. Nevertheless, this offset is within the limits of what could be expected if both species have different salinity threshold tolerances and the morphological change was triggered by salinity. Conversely, under the assumption that abundance is a good indicator for stress, it is unlikely that stress and individual fitness alone influences the population morphology, because during the first abundance drop in *O. universa* at the end of Interval 1 no morphological deviation could be observed. *Orbulina universa* seems to be able to resist salinity changes rather long, but after a certain threshold is reached shows heavy reactions, as evidenced by the later onset of morphological change and earlier local extinction. In contrast, *Globigerinoides sacculifer* seems to react earlier to the environmental stress levels, but due to this higher evolvability being better able to adapt to the changing environment and survive longer under equally unfavourable conditions. We thus hypothesize that the reactions seen in both species are a morphological reaction to stress induced by salinity changes, and that the abiotic and biotic component are so closely related that they cannot be disentangled. Conversely, species abundance seems to be a less valuable indicator of environmental stress, and might be more reliable in some species than in others.

While the *O. universa* community reacted towards the environmental stress by decanalization, we see massive evidence for canalization in *G. sacculifer* within several traits, which are either all selected for or which are integrated and cannot vary independently. While results for *O. universa* shell size and roundness are in line with observations from a Mediterranean Sapropel [22], the conservative bet-hedging observed there and evidenced by increasing abundances of abnormal morphotypes shortly before local extinctions could not be replicated here. This, however, can result from the different stress pattern in the Red Sea in comparison to the study by Weinkauf, Moller, Koch et al. [22]. Bet-hedging is hypothesized to be especially beneficial for a population that is exposed to a very variable environment which changes unpredictably. While this assumption is reasonable for the study presented in Weinkauf, Moller, Koch et al. [22], our study was explicitly chosen to investigate the effect

of one parameter that changes gradually. There is no reason to assume that the salinity in the Red Sea at that time changed unpredictably, rather than increasing continuously. Since the environment thus developed toward an unfavourable but not unstable state, there is no reason to expect bet-hedging in the community.

It is, however, interesting that a reduction in shell roundness in *O. universa* was already observed by Weinkauf, Moller, Koch et al. [22], but was there (in contrast to this study) associated with a decrease in salinity. This lends evidence to the hypothesis that some morphological parameters are indeed a neutral proxy for environmental stress, widely independent of the exact type of stressor or environmental forcing causing that stress.

While we thus have to admit that the direct correlation between environmental and biotic parameters and morphological deviation are hard to grasp and need further research, we could show that both communities showed clear morphological trends with increasing stress levels. It could not be clearly shown whether salinity changes as abiotic parameter or increasing stress levels were the main inductor for the observed morphological deviations, because both parameters are still too strictly coupled. The fact that morphological deviations in both species occurred relatively synchronous, however, makes it reasonable that salinity changes were a major influential parameter. Especially when assuming that *O. universa* showed no morphological deviation at the end of Interval 1, when the abundance already dropped significantly a first time. The results strongly suggest that morphology is a versatile tool to reconstruct past stress levels on the community, and can lead to better estimates about the timing of stress induction than species abundance (and thus population dynamics) can do.

## Supporting Information

**File S1.pdf Detailed summary of additional analytical results.** Unimodality in morphological parameters of *O. universa*, shape–size relationship in *G. sacculifer*. Error calculations.

**File S2.xlsx Relative and absolute abundance of *O. universa* and *G. sacculifer*.**

**File S3.xlsx Morphological parameters of *O. universa*.**

**File S4.tps Landmark data of *G. sacculifer*.**

## Acknowledgements

We thank the members of the micropa-laeontology workgroup at the MARUM Bremen and Minigraduiertenkolleg Tübingen for fruitful discussions concerning results of this study.

## Author Contributions

Conceived and designed the experiments: MFGW MK. Performed the experiments: MFGW KB FB MK. Analysed the data: MFGW MK. Contributed reagents/ materials/analysis tools: MFGW KB FB MK. Wrote the paper: MFGW KB FB MK.

## References

1. Moritz C and Agudo R (2013) The future of species under climate change: Resilience or decline? Science 341: 504–508. Doi: 10.1126/science.1237190.
2. Drake JM and Griffen BD (2010) Early warning signals of extinction in deteriorating environments. Nature 467: 456–459. Doi: 10.1038/nature09389.
3. Ludwig D (1999) Is it meaningful to estimate a probability of extinction? Ecology 80: 298–310. Doi: 10.1890/0012-9658(1999)080[0298:IIMTEA]2.0.CO;2.
4. Furlow FB, Armijo-Prewitt T, Gangestad SW and Thornhill R (1997) Fluctuating asymmetry and psychometric intelligence. Proc R Soc B 264: 823–829. Doi: 10.1098/rspb.1997.0115.
5. Leung B, Forbes MR and Houle D (2000) Fluctuating asymmetry as a bioindicator of stress: Comparing efficacy of analyses involving multiple traits. Am Nat 155: 101–115. Doi: 10.1086/303298.
6. Hendrickx F, Maelfait JP and Lens L (2003) Relationship between fluctuating asymmetry and fitness within and between stressed and unstressed populations of the wolf spider *Pirata piraticus*. J Evol Biol 16: 1270–1279. Doi: 10.1046/j.1420-9101.2003.00633.x.

7. Lens L, Van Dongen S, Kark S and Matthysen E (2002) Fluctuating asymmetry as an indicator of fitness: Can we bridge the gap between studies? Biol Rev Cambridge Philos Soc 77: 27–38. Doi: 10.1017/S1464793101005796.
8. Osterauer R, Marschner L, Betz O, Gerberding M, Sawasdee B, et al. (2010) Turning snails into slugs: induced body plan changes and formation of an internal shell. Evol Dev 12: 474–483. Doi: 10.1111/j.1525-142X.2010.00433.x.
9. Marschner L, Staniek J, Schuster S, Triebskorn R and Köhler H-R (2013) External and internal shell formation in the ramshorn snail *Marisa cornuarietis* are extremes in a continuum of gradual variation in development. BMC Dev Biol 13: Article 22. Doi: 10.1186/1471-213X-13-22.
10. Klingenberg CP (2003) A developmental perspective on developmental instability: Theory, models and mechanisms. In: M. Polak, editor. Developmental Instability: Causes and Consequences. New York: Oxford University Press. pp. 14–34.
11. Debat V and David P (2001) Mapping phenotypes: Canalization, plasticity and developmental stability. Trends Ecol Evol 11: 555–561. Doi: 10.1016/S0169-5347(01)02266-2.
12. Willmore KE, Klingenberg CP and Hallgrímsson B (2005) The relationship between fluctuating asymmetry and environmental variance in rhesus macaque skulls. Evol: Int J Org Evol 59: 898–909. Doi: 10.1111/j.0014-3820.2005.tb01763.x.
13. Van Valen L (1965) Morphological variation and width of ecological niche. Am Nat 99: 377–390.
14. Bull JJ (1987) Evolution of phenotypic variance. Evol: Int J Org Evol 41: 303–315.
15. Schmalhauzen II (1949) Factors of Evolution: The Theory of Stabilizing Selection. Madison: Blakiston Company. 327 p.
16. Sánchez-Chardi A, García-Pando M and López-Fuster MJ (2013) Chronic exposure to environmental stressors induces fluctuating asymmetry in shrews inhabiting protected Mediterranean sites. Chemosphere 93: 916–923. Doi: 10.1016/j.chemosphere.2013.05.056.
17. Takahashi KH, Daborn PJ, Hoffmann AA and Takano-Shimizu T (2011) Environmental stress-dependent effects of deletions encompassing *Hsp70Ba* on canalization and quantitative trait asymmetry in *Drosophila melanogaster*. PLOS ONE 6: e17295. Doi: 10.1371/journal.pone.0017295.
18. O'Keefe FR, Binder WJ, Frost SR, Sadlier RW and Van Valkenburgh B (2014) Cranial morphometrics of the dire wolf, *Canis dirus*, at Rancho La Brea: Temporal variability and its links to nutrient stress and climate. Palaeontol Electron 17: 17A.
19. Smith PL, Longridge LM, Grey M, Zhang J and Liang B (2014) From near extinction to recovery: Late Triassic to Middle Jurassic ammonoid shell geometry. Lethaia 47: 337–351. Doi: 10.1111/let.12058.
20. Hoffmann AA and Woods R (2001) Trait variability and stress: Canalization, developmental stability and the need for a broad approach. Ecol Lett 4: 97–101. Doi: 10.1046/j.1461-0248.2001.00205.x.
21. Santos M, Iriarte PF and Céspedes W (2005) Genetics and geometry of canalization and developmental stability in *Drosophila subobscura*. BMC Evol Biol 5: Article 7. Doi: 10.1186/1471-2148-5-7.
22. Weinkauf MFG, Moller T, Koch MC and Kučera M (2014) Disruptive selection and bet-hedging in planktonic Foraminifera: Shell morphology as predictor of extinctions. Front Ecol Evol 2: Article 64. Doi: 10.3389/fevo.2014.00064.
23. Fenton M, Geiselhart S, Rohling EJ and Hemleben C (2000) Aplanktonic zones in the Red Sea. Mar Micropaleontol 40: 277–294. Doi: 10.1016/S0377-8398(00)00042-6.
24. Kučera M (2007) Planktonic Foraminifera as tracers of past oceanic environments. In: C. Hillaire-Marcel, A. de Vernal and H. Chamley, editors. Proxies in Late Cenozoic Paleoceanography. Amsterdam: Elsevier. pp. 213–262.
25. Rohling EJ, Grant K, Bolshaw M, Roberts AP, Siddall M, et al. (2009) Antarctic temperature and global sea level closely coupled over the past five glacial cycles. Nat Geosci 2: 500–504. Doi: 10.1038/ngeo557.
26. Malmgren BA (1984) Analysis of the environmental influence on the morphology of *Ammonia beccarii* (LINNÉ) in Southern European salinas. Geobios 17: 737–746.
27. Malmgren BA and Kennett JP (1976) Biometric analysis of phenotypic variation in recent *Globigerina bulloides* d'Orbigny in the southern Indian Ocean. Mar Micropaleontol 1: 3–25. Doi: 10.1016/0377-8398(76)90003-7.
28. Moller T, Schulz H and Kučera M (2013) The effect of sea surface properties on shell morphology and size of the planktonic foraminifer *Neogloboquadrina pachyderma* in the North Atlantic. Palaeogeogr, Palaeoclimatol, Palaeoecol 391: 34–48. Doi: 10.1016/j.palaeo.2011.08.014.
29. Mary Y and Knappertsbusch MW (2013) Morphological variability of menardiform globorotalids in the Atlantic Ocean during Mid-Pliocene. Mar Micropaleontol 101: 180–193. Doi: 10.1016/j.marmicro.2012.12.001.
30. Nellen W, Bettac W, Roether W, Schnack D, Thiel H, et al. (1996) MINDIK Reise Nr. 5. Hamburg: Leitstelle METEOR. 179 p.

31. Amante C and Eakins BW (2009) ETOPO1 1 arc-minute global relief model: Procedures, data sources and analysis. National Geophysical Data Center. pp. 19.
32. Thunell RC, Locke SM and Williams DF (1988) Glacio-eustatic sea-level control on Red Sea salinity. Nature 334: 601–604. Doi: 10.1038/334601a0.
33. Rostek F, Bard E, Beaufort L, Sonzogni C and Ganssen G (1997) Sea surface temperature and productivity records for the past 240 kyr in the Arabian Sea. Deep-Sea Res, Part II 44: 1461–1480. Doi: 10.1016/S0967-0645(97)00008-8.
34. Prell WL, Martin A, Cullen JL and Trend M (1999) The Brown University Foraminiferal Data Base (BFD). Database: PANGAEA. http://doi.pangaea.de/10.1594/PANGAEA.96900.
35. Prell WL, Martin A, Cullen JL and Trend M (1999) The Brown University Foraminiferal Data Base. IGBP PAGES/World Data Center-A for Paleoclimatology Data Contribution Series. Boulder: NOAA/NGDC Paleoclimatology Program.
36. Schindelin J, Arganda-Carreras I, Frise E, Kayning V, Longair M, et al. (2012) Fiji: An open-source platform for biological-image analysis. Nat Methods 9: 676–682. Doi: 10.1038/nmeth.2019.
37. Caron DA, Faber WF, Jr. and Bé AWH (1987) Growth of the spinose planktonic foraminifer *Orbulina universa* in laboratory culture and the effect of temperature on life processes. J Mar Biol Assoc U K 67: 343–358. Doi: 10.1017/S0025315400026655.
38. R Development Core Team (2014) R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing.
39. André A, Weiner A, Quillévéré F, Aurahs R, Morard R, et al. (2013) The cryptic and the apparent reversed: Lack of genetic differentiation within the morphologically diverse plexus of the planktonic foraminifer *Globigerinoides sacculifer*. Paleobiology 39: 21–39. Doi: 10.5061/dryad.rb06j.
40. Shapiro SS and Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52: 591–611. Doi: 10.1093/biomet/52.3-4.591.
41. Fligner MA and Killeen TJ (1976) Distribution-free two-sample tests for scale. J Am Stat Assoc 71: 210–213. Doi: 10.1080/01621459.1976.10481517.
42. Davison AC and Hinkley DV (1997) Bootstrap Methods and their Applications. Cambridge: Cambridge University Press. 582 p.
43. Tabor J (2010) Investigating the investigative task: Testing for skewness—an investigation of different test statistics and their power to detect skewness. J Stat Educ 18: 1–13.
44. Tabachnick BG and Fidell LS (1996) Using Multivariate Statistics. New York: Harper Collins. 880 p.
45. Dixon PM (2002) Bootstrap resampling. In: A. H. El-Shaarawi and W. W. Piegorsch, editors. Encyclopedia of environmetrics. Chichester: John Wiley & Sons, Ltd. pp. 212–220.
46. Heslop D, De Schepper S and Proske U (2011) Diagnosing the uncertainty of taxa relative abundances derived from count data. Mar Micropaleontol 79: 114–120. Doi: 10.1016/j.marmicro.2011.01.007.
47. Sheskin DJ (2011) Handbook of Parametric and Nonparametric Statistical Procedures. Boca Raton, London, New York: Chapman & Hall/CRC Press. 1886 p.
48. Kruskal WH and Wallis WA (1952) Use of ranks in one-criterion variance analysis. J Am Stat Assoc 47: 583–621. Doi: 10.1080/01621459.1952.10483441.
49. Mann HB and Whitney DR (1947) On a test of whether one of two random variables is stochastically larger than the other. Ann Math Stat 18: 50–60. Doi: 10.1214/aoms/1177730491.
50. Benjamini Y and Hochberg Y (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc, Ser B: Met 57: 289–300. Doi: 10.2307/2346101.
51. Reuss AE (1850) Neue Foraminiferen aus den Schichten des österreichischen Tertiärbeckens. Denkschr Kaiserl Akad Wiss, Math–Naturwiss Classe 1: 365–390.
52. Banner FT and Blow WH (1960) Some primary types of species belonging to the superfamily Globigerinaceae. Contrib Cushman Found Foraminiferal Res 11: 1–41.
53. Hartigan JA and Hartigan PM (1985) The dip test of unimodality. Ann Stat 13: 70–84. Doi: 10.1214/aos/1176346577.
54. Claude J (2008) Morphometrics with R. New York: Springer-Verlag. 316 p.
55. Bookstein FL (1991) Morphometric tools for landmark data: Geometry and biology. Cambridge, New York, Port Chester, Melbourne, Sydney: Cambridge University Press. 455 p.
56. Zelditch ML, Swiderski DL and Sheets HD (2012) Geometric Morphometrics for Biologists: A Primer. London, Waltham, San Diego: Academic Press. 478 p.
57. Mosimann JE (1970) Size allometry: Size and shape variables with characterizations of the lognormal and generalized gamma distributions. J Am Stat Assoc 65: 930–945. Doi: 10.2307/2284599.
58. Hunt G (2006) Fitting and comparing models of phyletic evolution: Random walks and

beyond. Paleobiology 32: 578–601. Doi: 10.1666/05070.1.

59. Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19: 716–723. Doi: 10.1109/TAC.1974.1100705.
60. Mevik B-H and Wehrens R (2007) The pls package: Principal component and partial least squares regression in R. J Stat Software 18: Article 2.
61. Kendall DG (1984) Shape manifolds, Procrustean metrics, and complex projective spaces. Bull London Math Soc 16: 81–121. Doi: 10.1112/blms/16.2.81.
62. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26: 32–46. Doi: 10.1111/j.1442-9993.2001.01070.pp.x.
63. Fisher RA (1936) The use of multiple measurements in taxonomic problems. Ann Hum Genet 7: 179–188. Doi: 10.1111/j.1469-1809.1936.tb02137.x.
64. Venables WN and Ripley BD (2002) Modern Applied Statistics with S. Springer-Verlag. 495 p.
65. Wagner GP and Altenberg L (1996) Perspective: Complex adaptations and the evolution of evolvability. Evol: Int J Org Evol 50: 967–976. Doi: 10.2307/2410639.
66. de Vargas C, Norris RD, Zaninetti L, Gibb SW and Pawlowski J (1999) Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces. Proc Natl Acad Sci USA 96: 2864–2868. Doi: 10.1073/pnas.96.6.2864.
67. Darling KF and Wade CM (2008) The genetic diversity of planktic Foraminifera and the global distribution of ribosomal RNA genotypes. Mar Micropaleontol 67: 216–238. Doi: 10.1016/j.marmicro.2008.01.009.
68. Morard R, Quillévéré F, Escarguel G, Ujiie Y, de Garidel-Thoron T, et al. (2009) Morphological recognition of cryptic species in the planktonic foraminifer *Orbulina universa*. Mar Micropaleontol 71: 148–165. Doi: 10.1016/j.marmicro.2009.03.001.
69. Bolker BM (2008) Ecological Models and Data in R. New Jersey, Woodstock: Princeton University Press. 396 p.
70. Doebeli M (1996) A quantitative genetic competition model for sympatric speciation. J Evol Biol 9: 893–909. Doi: 10.1046/j.1420-9101.1996.9060893.x.
71. Wagner GP (1996) Homologues, natural kinds and the evolution of modularity. Am Zool 36: 36–43.
72. Klingenberg CP (2008) Morphological integration and developmental modularity. Annu Rev Ecol Evol Syst 39: 115–132. Doi: 10.1146/annurev.ecolsys.37.091305.110054.
73. Sofianos SS and Johns WE (2003) An Oceanic General Circulation Model (OGCM) investigation of the Red Sea circulation: 2. Three-dimensional circulation in the Red Sea. J Geophys Res, Oceans 108: Article 3066. Doi: 10.1029/2001JC001185.
74. Haenel P (1987) Intérêt paléoocéanographique d'*Orbulina universa* d'Orbigny (foraminifère). Oceanol Acta 10: 15–25.
75. Spero HJ (1988) Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*. Mar Biol 99: 9–20. Doi: 10.1007/BF00644972.
76. Ortiz JD, Mix AC and Collier RW (1995) Environmental control of living symbiotic and asymbiotic Foraminifera of the California Current. Paleoceanography 10: 987–1009. Doi: 10.1029/95PA02088.
77. Schmidt DN, Renaud S, Bollmann J, Schiebel R and Thierstein HR (2004) Size distribution of Holocene planktic foraminifer assemblages: Biogeography, ecology and adaptation. Mar Micropaleontol 50: 319–338. Doi: 10.1016/S0377-8398(03)00098-7.
78. Vangel MG (1996) Confidence intervals for a normal coefficient of variation. Am Stat 50: 21–26.
79. Waddington CH (1942) Canalization of development and the inheritance of aquired characters. Nature 150: 563–565. Doi: 10.1038/150563a0.
80. Pélabon C, Hansen TF, Carter AJR and Houle D (2010) Evolution of variation and variability under fluctuating, stabilizing, and disruptive selection. Evol: Int J Org Evol 64: 1912–1925. Doi: 10.1111/j.1558-5646.2010.00979.x.
81. Kawecki TJ (2000) The evolution of genetic canalization under fluctuating selection. Evol: Int J Org Evol 54: 1–12. Doi: 10.1111/j.0014-3820.2000.tb00001.x.
82. Van Valen L (1962) A study of fluctuating asymmetry. Evol: Int J Org Evol 16: 125–142.
83. Brummer GJA and Kroon D (1988) Planktonic Foraminifers as Tracers of Ocean-Climate History: Ontogeny, Relationships and Preservation of Modern Species and Stable Isotopes, Phenotypes and Assemblage Distribution in Different Water Masses. Amsterdam: Free University Press. 346 p.
84. Timofeéf-Ressovsky NW (1934) Über den Einfluss des genetischen Milieus und der Aussenbedingungen auf die Realisation des Genotyps. Nachrichten Gesellsch Wissensch Göttingen, Math–Physikalische Kl 6: 53–104.
85. Graham JH, Emlen JM, Freeman DC, Leamy LJ and Kieser JA (1998) Directional asymmetry and the measurement of developmental instability. Biol J Linn Soc 64: 1–16. Doi: 10.1111/j.1095-8312.1998.tb01530.x.

86. Graham JH, Freeman DC and Emlen JM (1993) Antisymmetry, directional asymmetry, and dynamic morphogenesis. Genetica 89: 121–137. Doi: 10.1007/BF02424509.

87. Hemleben C, Spindler M, Breitinger I and Ott R (1987) Morphological and physiological responses of *Globigerinoides sacculifer* (Brady) under varying laboratory conditions. Mar Micropaleontol 12: 305–324. Doi: 10.1016/0377-8398(87)90025-9.

88. Breuker CJ, Patterson JS and Klingenberg CP (2006) A single basis for developmental buffering of *Drosophila* wing shape PLOS ONE 1: e7. Doi: 10.1371/journal.pone.0000007.

89. Klingenberg CP, Duttke S, Whelan S and M. K (2012) Developmental plasticity, morphological variation and evolvability: A multilevel analysis of morphometric integration in the shape of compound leaves. J Evol Biol 25: 115–129. Doi: 10.1111/j.1420-9101.2011.02410.x.

90. Breno M, Leirs H and Van Dongen S (2011) No relationship between canalization and developmental stability of the skull in a natural population of *Mastomys natalensis* (Rodentia: Muridae). Biol J Linn Soc 104: 207–216. Doi: 10.1111/j.1095-8312.2011.01702.x.

# Part III

# Additional material

# Curriculum vitae

## Personal Data

| | |
|---|---|
| Full name | Manuel Fritz Gerhard Weinkauf |
| Nationality | German |
| Date of birth | 31 May 1983 |
| Place of birth | Rüdersdorf bei Berlin |

## Education and Qualifications

| | |
|---|---|
| 3 March 2013–29 March 2013 | Transit cruise DipFIP with RV Sonne, Wellington–Kaohsiung |
| 15 July 2012–21 July 2012 | 'Field Workshop on Living Foraminifera in Japan', Sesoko (Okinawa) and Yokosuka, organized by Hiroshi Kitazato et al. |
| Since 1 Jan. 2011 | Ph.D. student at the Eberhard–Karls-Universität Tübingen, 'Morphological variability and symmetry of foraminiferal shells in response to rapid environmental change', Supervisors: Prof. Dr Michal Kučera and Prof. Dr Heinz-R. Köhler |
| 11 Oct. 2010 | Received title of *Diplom Geologe* (equivalent to M.Sc. in Geosciences) from the Freie Universität Berlin: Exams in 'General Geology', 'Historical and Regional Geology', 'Palaeontology', and 'Zoology' |

| | |
|---|---|
| 15 Dec. 2008–23 Jan. 2009 | Museum für Naturkunde Berlin, student practical supervised by Dr David Lazarus—data analysis and method-development for palaeobiological data |
| 10 Sept. 2007–5 Oct. 2007 | Naturhistorisches Museum/Landessammlung für Naturkunde Rheinland–Pfalz, student practical supervised by Dipl.-Geol. Uwe Kaulfuß—excavation in the Eckfelder Maar |
| 11 Sept. 2006–11 Oct. 2008 | Wall Street Institute, course to enhance command of English |
| 1 Oct. 2003–30 Sept. 2010 | Freie Universität Berlin, studying 'Geology and Palaeontology' |
| 28 June 2002 | Carl Bechstein Gymnasium Erkner, *Abitur* (A levels) |
| 1999–2002 | Carl Bechstein Gymnasium Erkner (secondary school) |
| 1995–1999 | Gesamtschule Neu Zittau (secondary school) |
| 1989–1995 | Grundschule Neu Zittau (primary school) |

## Grants and Awards

| | |
|---|---|
| 1 June 2013 | Johanna M. Resig Foraminiferal Research Fellowship of the Cushman Foundation, US-$ 25 000 to proceed and enhance work on Ph.D. project |
| 2012 | The Micropalaeontological Society Grant-in-Aid, £300 to cover travel expenses for presenting at the Micropalaeontological Society Foraminifera and Nannofossil Groups Joint Meeting 2012 in Edinburgh |
| 1 Jan. 2011–31 Dec. 2013 | Landesministerium für Forschung, Bildung und Kunst Baden–Württemberg, LGFG stipend for Ph.D. project |

## Teaching experience

| | |
|---|---|
| winter 2013–spring 2014 | Tutoring in a course concerned with quantitative analyses in palaeontology |
| spring 2013 | Tutoring one student in a short course/practical: quantitatively assessing calcification intensity in planktonic Foraminifera |
| winter 2012–spring 2013 | Tutoring in a course concerned with quantitative analyses in palaeontology |
| spring 2012 | Tutoring two students in a short course/practical: traditional morphometrics applied to fossil brachiopods |

## Employment

| | |
|---|---|
| 1 July 2002–30 Apr. 2003 | *Zivildienst* (alternative civilian service) at an old people's home, Seniorenwohnheim Erkner GmbH, Marseille Kliniken AG |

# Publication list

## Peer reviewed articles

Weiner, A. K. M., Weinkauf, M. F. G., Kurasawa, A., Darling, K. F., and Kučera, M. (2015) Genetic and morphometric evidence for parallel evolution of the *Globigerinella calida* morphotype, *Marine Micropaleontology* 114: 19–35, doi:10.1016/j.marmicro.2014.10.003.

Weiner, A. K. M., Weinkauf, M. F. G., Kurasawa, A., Darling, K. F., Kučera, M., and Grimm, G. W. (2014) Phylogeography of the tropical planktonic Foraminifera lineage *Globigerinella* reveals isolation inconsistent with passive dispersal by ocean currents, *PLOS ONE* 9 (3): e92148, doi:10.1371/journal.pone.0092148.

Weinkauf, M. F. G., Moller, T., Koch, M. C., and Kučera, M. (2014) Disruptive selection and bet-hedging in planktonic Foraminifera: Shell morphology as predictor of extinctions, *Frontiers in Ecology and Evolution* 2: Article 64, doi:10.3389/fevo.2014.00064.

Milker, Y., Rachmayani, R., Weinkauf, M. F. G., Prange, M., Raitzsch, M., Schulz, M., and Kučera, M. (2013) Global and regional sea surface temperature trends during Marine Isotope Stage 11, *Climate of the Past* 9 (5): 2231–52, doi:10.5194/cp-9-2231-2013.

Weinkauf, M. F. G., Keupp, H., and Mutterlose, J. (2013) Calcareous dinoflagellates from the Late Hauterivian (Early Cretaceous) of Frielingen, Germany, *Documenta naturae* 192 (3): 241–71, http://www.researchgate.net/publication/246548209_Calcareous_dinoflagellates_from_the_Late_Hauterivian_(Early_Cretaceous)_of_Frielingen_Germany.

Weinkauf, M. F. G., Moller, T., Koch, M. C., and Kučera, M. (2013) Calcification intensity in planktonic Foraminifera reflects ambient conditions irrespective of environmental stress, *Biogeosciences* 10 (10): 6639–55, doi:10.5194/bg-10-6639-2013.

Lazarus, D., Weinkauf, M., and Diver, P. (2012) Pacman Profiling: A simple procedure to identify stratigraphic outliers in high-density deep-sea microfossil data, *Paleobiology* 38 (1): 144–61, doi:10.1666/10067.1.

## Book chapters

Milker, Y., Rachmayani, R., Weinkauf, M. F. G., Prange, M., Raitzsch, M., Schulz, M., and Kučera, M. (2015) Global synthesis of sea surface temperature trends during Marine Isotope Stage 11, In Schulz, M. and Paul, A. (eds.), *Integrated Analysis of Interglacial Climate Dynamics* (INTERDYNAMIC), SpringerBriefs in Earth System Sciences, (Cham,

Heidelberg, New York, Dordrecht, London: Springer-Verlag), pp. 13–18, doi:10.1007/978-3-319-00693-2_3.

## Other articles

Kučera, M., Morard, R., Siccha, M., Weiner, A., and Weinkauf, M. (2013) Cruise report of RV Sonne Cruise SO226-3 DipFIP – the extent and structure of cryptic diversity in morphospecies of planktonic Foraminifera of the Indopacific Warm Pool: Wellington–Kaohsiung, 04.03.2013–28.03.2013, *Berichte aus dem MARUM und dem Fachbereich Geowissenschaften der Universiät Bremen* 293: 1–39, http://elib.suub.uni-bremen.de/edocs/00103212-1.pdf.

Fox, L. and Weinkauf, M. (2012) The Micropalaeontology Society Foraminifera and Nannofossil Groups joint meeting 2012, 21–23 June 2012, Edinburgh, Scotland, United Kingdom, *Newsletter of Micropalaeontology* 86: 27–30, http://www.tmsoc.org/newsletter.htm.

## Conference contributions

Weinkauf, M. F. G., Kunze, J. G., and Kučera, M. (2014) Foraminifera shell calcification, In Edgar, K. and Austin, B. (eds.), MORPHOMETRICS *10: Identifying the Top 10 Questions in Morphometrics and Micropalaeontology Today*, (Texel: The Micropalaentological Society), (Talk).

Weinkauf, M. F. G., Moller, T., Koch, M. C., and Kučera, M. (2014) Evidence for disruptive and stabilizing selection and bet-hedging in pre-extinction populations of planktonic Foraminifera, In Ufkes, E., Brummer, G.-J., de Nooijer, L., Reichart, G.-J., and Peeters, F. (eds.), *Foraminifera and Nannofossil Groups Joint Meeting 2014: Foraminifera and Nannofossils through Time; Qualification and Quantification*, (Texel: The Micropalaentological Society), p. 46, (Poster).

Weinkauf, M. F. G., Moller, T., Koch, M. C., and Kučera, M. (2013) Calcification intensity in planktonic Foraminifera reflects ambient conditions irrespective of environmental stress, In Scheil, A., Scheil, V., Triebskorn, R., and Köhler, H.-R. (eds.), *Meeting StEvE 24.–25. October 2013*, (Tübingen), (Poster).

Weinkauf, M., Moller, T., Koch, M., and Kučera, M. (2013) Calcification intensity in planktonic Foraminifera reflects ambient conditions irrespective of environmental stress, In Holcová, K., Bubík, M., Švábenická, L., and Vodrážka, R. (eds.), *The Micropalaeontological Society Foraminifera and Nannofossil Groups Spring Meeting 2013, Prague, Czech Republic, 19th–22nd June, 2013—The Micropalaeontological Record of Global Change: From Epicontinental Seas to Open Oceans*, The Micropalaeontological Society, (Prague), p. 41, (Talk).

Weinkauf, M., Moller, T., Koch, M., and Kučera, M. (2012) Effects of environmental change on the calcification intensity of planktonic Foraminifera: A case study from Sapropel S5, *Terra Nostra* 3, Centenary Meeting of the Paläontologische Gesellschaft 2012: 192–3, http://palaeo100.naturkundemuseum-berlin.de/fileadmin/startseite/palaeo100/Tagungsband.pdf, (Talk).

Weinkauf, M., Moller, T., Koch, M., and Kučera, M. (2012) Effects of environmental change on the calcification intensity of planktonic Foraminifera: A case study from Sapropel S5, In *Meeting StEvE 2012*, (Tübingen), p. 4, http://www.uni-tuebingen.de/en/faculties/faculty-of-science/departments/biologie/institute/evolutionecology/groups/animal-evolutionary-ecology/eve/meeting-steve.html, (Talk).

Weinkauf, M., Moller, T., Koch, M., and Kučera, M. (2012) Morphological deviations in planktonic Foraminifera as a reaction to enhanced environmental stress: Opportunities and problems, *Terra Nostra* 3, Centenary Meeting of the Paläontologische Gesellschaft 2012: 193–4, http://palaeo100.naturkundemuseum-berlin.de/fileadmin/startseite/palaeo100/Tagungsband.pdf, (Poster).

Weinkauf, M., Moller, T., Koch, M., and Kučera, M. (2012) Morphological response of Foraminifera to environmental stress during the deposition of a Mediterranean sapropel, In Darling, K., Evans, K., Bird, C., Schweizer, M., and Russon, T. (eds.), *The Micropalaeontological Society Foraminifera and Nannofossil Groups Joint Meeting 2012*, The Micropalaeontological Society, (Edinburgh), pp. 47–8, (Talk).

Weinkauf, M., Moller, T., Koch, M., and Kučera, M. (2012) Morphological response of Foraminifera to environmental stress during the deposition of a Mediterranean sapropel, In Kitazato, H. (ed.), *Field Workshop on Living Foraminifera in Japan*, JAMSTEC, Institute of Biogeosciences, (Sesoko, Yokosuka), pp. 43–4, (Talk).

Weinkauf, M., Moller, T., Koch, M., and Kučera, M. (2011) Morphological response of Foraminifera to environmental stress, In *StEvE Meeting 2–3 December 2011*, (Tübingen), p. 9, http://www.geo.uni-tuebingen.de/aktuelles/forschungskolloquien-und-seminare/steve-meeting.html, (Talk).

Weinkauf, M., Moller, T., Koch, M., and Kučera, M. (2011) Morphological variation of *Orbulina universa* in response to environmental change (Sapropel S5, Eastern Mediterranean Sea), In Bąk, M., Kaminski, M. A., and Waśkowska, A. (eds.), *Integrating Microfossil Records from the Oceans and Epicontinental Seas*, Special Publications 17, The Micropalaeontological Society Foraminifera and Nannofossil Groups Joint Meeting 2011, The Micropalaeontological Society, (Krakow: The Grzybowski Foundation), p. 142, http://gf.tmsoc.org/Publications-main.html, (Poster).

## Theses

Weinkauf, M. (2010) 'Calcareous Dinoflagellate Cysts from the Upper Hauterivian of the Clay Pit Frielingen (NW Germany) and their Palaeoecological Implications', Diploma thesis, Berlin: Freie Universität Berlin, 120 pp.

Weinkauf, M. (2010) 'Comments on the Geological Map of the Breitenberg Area (Northern Calcareous Alps, Austria)', Diploma mapping report, Berlin: Freie Universität Berlin, 89 pp.