Reassessing Foraminiferal Stable Isotope Geochemistry: Impact of the Oceanic Carbonate System (Experimental Results)

J. Bijma[1*], H.J. Spero[2] and D.W. Lea[3]

[1]*Universität Bremen, Fachbereich Geowissenschaften, Postfach 33 04 40, D-28334 Bremen, Germany*
[2]*Department of Geology, University of California Davis, Davis, California 95616, U.S.A.*
[3]*Department of Geological Sciences and the Marine Science Institute, University of California Santa Barbara, Santa Barbara, California 93106, U.S.A.*
** corresponding author (e-mail): jbijma@uni-bremen. de*

Abstract: Laboratory experiments with living planktic foraminifers show that the $\delta^{13}C$ and $\delta^{18}O$ values of shell calcite decrease with increasing sea water pH and/or carbonate ion concentration. The effect has been quantified in symbiotic (*Orbulina universa*) and non-symbiotic (*Globigerina bulloides*) species and is independent of symbiont activity and temperature. It is concluded that a kinetic fractionation process affects both the carbon and oxygen isotopic composition of the shell simultaneously. At present it cannot be determined definitively whether the relationship is controlled by the pH dependent balance between hydration and hydroxylation of CO_2 or by $[CO_3^{2-}]$ related variations in the calcification rate. However, independent of which factor ultimately controls the relationship between the carbonate chemistry and isotopic fractionation, in the real ocean $[CO_3^{2-}]$ and pH covary linearly across the relevant pH range. The true relationship between shell isotopic composition and the bulk carbonate chemistry is masked by the fact that host respiration and symbiont activity locally modify the carbonate system. Respiration lowers and photosynthesis increases ambient pH and $[CO_3^{2-}]$. This translates into modified absolute shell values but leaves the slope between the shell isotopic composition and the bulk carbonate chemistry unaffected. A second level of shell isotopic modification is introduced by the incorporation of respired carbon, enriched in ^{12}C, which depletes the shell $\delta^{13}C$ value. In symbiont bearing species this depletion is partially negated by a shell $\delta^{13}C$ enrichment in the light. As an alternative to the RUBISCO hypothesis (enrichment via preferential removal of $^{12}CO_2$), we propose that scavenging of respired CO_2 during photosynthesis, raises the shell $\delta^{13}C$ value. Our results have partly been documented before (Spero et al. 1997) and demonstrate that the carbonate chemistry is undoubtedly a major control on temporal geochemical variability in the fossil record. For instance, the sea water carbonate system of the pre-Phanerozoic world (Berner 1994; Grotzinger and Kasting 1993) or during glacials (Sanyal et al. 1995) was significantly different from today confounding direct interpretation of foraminiferal stable isotope data using existing relationships (see companion paper in this volume by Lea et al.).

Introduction

Spero et al. (1997) describe a series of experiments demonstrating that planktic foraminiferal $\delta^{18}O$ and $\delta^{13}C$ are influenced by sea water carbonate chemistry. In this paper, we describe these experiments in greater detail, discuss previously unpublished experiments and speculate on the potential mechanisms controlling the observed responses.

Based on a theoretical study of the thermodynamic properties of isotopic substances, Urey (1947) proposed that the ^{18}O content of calcium carbon-

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ate could be used as a paleothermometer for the ocean. The method is based on the fact that sea water and calcite differ in their $^{18}O/^{16}O$ ratios, when in thermodynamic equilibrium, and that this difference decreases with increasing temperature. McCrea (1950) demonstrated in the laboratory that synthetic calcium carbonate when precipitated in equilibrium with sea water is enriched in ^{18}O relative to sea water but less so with increasing temperature and thus behaves as predicted by Urey. This empirical relationship was the first, so called, paleotemperature relationship. Another milestone were the papers by Epstein et al. (1951, 1953) who were the first to show that marine invertebrates grown in the natural environment deposit calcium carbonate in equilibrium with sea water. Many new paleotemperature equations have been developed since this early pioneer work (for a review see Bemis et al. 1998) but the problem in determining the "true" paleotemperature is not only one of choosing the correct equation but, more importantly, one of choosing the correct value for $\delta^{18}O$ of sea water ($\delta^{18}O_w$). For the geologic past, this amounts to knowing paleosalinity and the relationship between salinity and $\delta^{18}O_w$ (e.g. Berger 1979; Broecker and Van Donk 1970; Fairbanks et al. 1992; Rozanski et al. 1993; Schrag et al. 1996; Shackleton 1967, 1974).

Beyond the glacial ice volume effect and the effect of precipitation/evaporation, so called "vital effects" were recognized. Contrary to Emiliani's (1954) interpretation that the oxygen isotopic differences among planktic foraminifers reflect differences in depth habitat and hence differences in precipitation temperature, Duplessy et al. (1970) argued that the differential oxygen isotopic fractionation in benthic foraminifers from the same level in a core requires a different explanation and they suggested that metabolic CO_2 depleted in ^{18}O is incorporated into the shells of benthic and possibly also planktic foraminifers. The hypothesis that planktic foraminifers have different ^{18}O-fractionation factors was subsequently confirmed by Shackleton et al. (1973) and later substantiated by numerous other studies (e.g. Curry and Matthews 1981a; Duplessy et al. 1981; Fairbanks et al. 1980; Shackleton 1974; Vergnaud-Grazzini 1976; Williams et al. 1979). For instance, Buchardt and Hansen (1977) note that benthic foraminifers bearing symbiotic algae are depleted in ^{18}O relative to symbiont barren species and Erez (1978b) argues that with greater photosynthetic activity, incorporation of light metabolic CO_2 results in anomalously light carbon and oxygen isotopic compositions of hermatypic corals and benthic foraminifers. However, when dealing with differences in $\delta^{18}O$ through time, vital effects have generally been assumed to be constant (or zero) and were therefore neglected.

The interpretation of the carbon isotopic composition proved to be more complicated than $\delta^{18}O$. Due to the preferential fixation of $^{12}CO_2$ by primary producers and subsequent transport and decomposition of organic matter below the euphotic zone, surface waters of the ocean are depleted in ^{12}C whereas the deeper ocean is ^{12}C-enriched. Thus, as a consequence of community production and respiration, $\delta^{13}C$ depth profiles are related to the oxygen concentration and inversely correlated with the nutrient concentration (the stoichiometric proportionality constant with PO_4 is ca. 0.93 ‰ per mol,kg^{-1}: Broecker and Peng 1982). Hence the $\delta^{13}C$ of surface dwelling foraminifers is used as a surface water fertility proxy and the difference in $\delta^{13}C$ between planktics and benthics ($\Delta\delta^{13}C$) was introduced as a measure of the strength of the biological pump (e.g. Broecker 1971; 1973; 1981). Another source for variation of $\delta^{13}C$ in dissolved inorganic carbon ($\delta^{13}C_{\Sigma CO2}$) is the fractionation between carbon in atmospheric CO_2 and the total dissolved CO_2 (ΣCO_2) in surface ocean water. The $^{13}C/^{12}C$ ratio in atmospheric CO_2 is on average 9 ‰ lower than that in surface ocean ΣCO_2 and the extent of fractionation depends on temperature (e.g. Broecker and Maier-Reimer 1992; Charles et al. 1993; Lynch-Stieglitz et al. 1995). As a result, high latitude surface water have a higher $^{13}C/^{12}C$ ratio than low latitude surface water. Generally, this thermodynamic imprint is not very strong due to very slow isotopic equilibration (Broecker and Peng 1982), however, if there is sufficient time for gas exchange, a significant signal is produced (Charles and Fairbanks 1990). A third source of variation is the so called "Mackensen effect".

Mackensen et al. (1993) hypothesised that growth and reproduction of benthic foraminifers coincides with the seasonal flux of phytodetritus. Because $\delta^{13}C$ of freshly accumulated phytodetritus was 3 to 4 ‰ lower than surface sediment organic carbon they argued that $\delta^{13}C$ of epibenthic shells, formed during remineralisation of this fluffy layer (locally and temporarily depleting $\delta^{13}C_{\Sigma CO2}$), is depleted compared to $\delta^{13}C_{\Sigma CO2}$ found throughout the rest of the year.

The main complication associated with the interpretation of $\delta^{13}C$ records from foraminiferal shells is a prominent vital effect. Already Craig (Revelle and Fairbridge 1957) had shown that the carbon isotopes in foraminiferal shells are precipitated out of equilibrium with $\delta^{13}C$ of ΣCO_2 and potentially reflect utilization of metabolic CO_2. The large range of disequilibrium precipitation was confirmed by many studies (for a review see e.g. Berger 1979; Duplessy 1978). A milestone for the investigation of vital effects on stable isotopic fractionation was the establishment of culture techniques for planktic foraminifers by the research groups of Allen Bé, Roger Anderson, Christoph Hemleben and Michael Spindler (e.g. Bé 1979; Bé 1980; Bé 1982; Bé and Anderson 1976; Bé et al. 1979; Bé et al. 1977; Hemleben and Spindler 1983; Hemleben et al. 1989; Hemleben et al. 1985; Hemleben et al. 1987; Spindler and Hemleben 1980). Using laboratory cultures, Erez and Luz (1983) derived the first empirical paleotemperature equation for *Globigerinoides sacculifer* and, for instance, Bouvier-Soumagnac and Duplessy (1985) were able to verify their plankton tow based temperature:$\delta^{18}O$ relationships. Culture experiments were also imperative to investigate the impact of algal symbiosis on the shell $\delta^{13}C$ composition of benthic (Erez 1978b; Williams et al. 1981b; Zimmermann et al. 1983) and planktic foraminifers (Spero and DeNiro 1987). In contrast to benthic foraminifers and some corals (Erez 1978b; Land et al. 1977), Spero and Deniro concluded that increased symbiont photosynthetic activity in *Orbulina universa*, resulted in a ^{13}C enrichment of the shell. Their conclusion was supported by other studies (Cummings and McCarty 1982; Goreau 1977; Weber and Woodhead 1970) and later verified for other species of planktic foraminifers (Bijma et al. 1998; Spero and Lea 1993; Spero et al. 1991). The enrichment was attributed to the preferential removal of $^{12}CO_2$ by the CO_2 fixing enzyme ribulose 1,5-biphosphate carboxylase-oxygenase (RUBISCO) during symbiont photosynthesis, enriching the calcifying environment in ^{13}C. Host respiration was shown to deplete the shell isotopic composition (e.g. Bijma et al. 1998; Spero and Lea 1996). This observation was explained by the contribution of metabolic CO_2, enriched in ^{12}C, to the calcifying environment.

During the past four decades, stable oxygen and carbon isotope measurements on biogenic calcite and aragonite (for a review see e.g. Wefer and Berger 1991) have become standard tools for reconstructing paleoceanographic and paleoclimatic change. By assuming that our understanding of the major parameters controlling stable isotope incorporation into biogenic calcite is complete, these geochemical proxies have been used to reconstruct glacial ice volumes (e.g. Fairbanks 1989; Mix 1987), sea surface (SST) and deep water temperatures (e.g. Zahn and Mix 1991), ocean circulation changes (e.g. Boyle 1990; Charles and Fairbanks 1992; Labeyrie et al. 1987) and glacial-interglacial shifts between the terrestrial and oceanic carbon pools (Shackleton 1977). However, the list of factors controlling the stable isotopic compositions of shells is still incomplete. Data from isotopic calibration studies have suggested that an unidentified parameter affects carbon and oxygen isotope ratios in foraminiferal shells (e.g. Duplessy et al. 1981; Fairbanks et al. 1982; Williams et al. 1979). Although shell $\delta^{13}C$ values may be affected by vital effects, $\delta^{18}O$ values should be insensitive to physiology. Yet, oxygen isotope data from foraminifers collected in surface plankton tows yield SST reconstructions that are several degrees warmer than measured ocean temperatures (Williams et al. 1981a). Similarly, the covariation of carbon and oxygen isotopes in planktic foraminifers (e.g. Berger et al. 1978; Curry and Matthews 1981a; Curry and Matthews 1981b; Kahn and Williams 1981) cannot be explained by shifting depth habitats during ontogeny. In terms of equilibrium fractionation the trends are opposite to those found

in the water column. If foraminifers were fractionating in isotopic equilibrium lightest $\delta^{18}O$ values should be associated with heaviest $\delta^{13}C$ values. Stable isotope studies on other calcifying organisms such as calcareous algae, hermatypic corals and echinoderms show similar disequilibria (McConnaughey 1989b; Wefer and Berger 1991) suggesting that these proxies are also equivocal and that some environmental or biological parameter(s) in addition to those already identified must be affecting skeletal stable isotope values. Interestingly, laboratory and field data show that skeletal $\delta^{18}O$ and $\delta^{13}C$ covary (McConnaughey 1989a; McConnaughey 1989b; Spero et al. 1997; Spero and Lea 1996) suggesting the mechanism that affects shell $^{18}O/^{16}O$ ratios also has an effect on $^{13}C/^{12}C$.

In this paper we examine the impact of changes in carbonate chemistry on the stable carbon and oxygen isotope composition of a symbiont bearing and a symbiont barren species of planktic foraminifers and subsequently discuss potential equilibrium and kinetic fractionation mechanisms. The implications of the observed isotope effects for paleo-oceanographic and -climatic reconstructions are discussed in the companion paper by Lea et al. (this volume).

Our initial hypothesis was that glacial symbiont bearing foraminifers should be depleted in ^{13}C relative to their Holocene counterparts because dissolved CO_2 availability (the CO_2aq concentration) was reduced in the glacial ocean. Since the $\delta^{13}C$ of algae increases as less CO_2aq is available (Farquhar et al. 1982) and [CO_2aq] was reduced in the glacial ocean, we predicted that symbiont fractionation and hence ^{13}C enrichment of the calcifying environment was reduced during glacial times. We found that symbiont bearing foraminifers grown at lower [CO_2aq] are indeed depleted in ^{13}C but for a different reason than we initially hypothesised.

Material and Methods

During the summers of 1993 through 1996 culture experiments were conducted at the Wrigley Environmental Science Center on Santa Catalina Island (California) to investigate the influence of sea water carbonate chemistry on the $\delta^{13}C$ and $\delta^{18}O$ of the shells of the symbiont bearing planktic foraminifer *Orbulina universa* (d'Orbigny) and the symbiont barren *Globigerina bulloides* d'Orbigny.

Orbulina Universa

Orbulina universa has a tropical to temperate distribution in the euphotic zone. Each adult individual is associated with 3,000 to 7,000 (Spero and Parker 1985) dinoflagellate symbionts (*Gymnodinium beii*). The symbionts are distributed in a halo around the calcitic shell (cf. Fig.1a). *Orbulina universa*, like most spinose species, is carnivorous and feeds primarily on calanoid copepods at a rate of one to two per day (Spindler et al. 1984). New chambers are added to the existing trochospire at regular intervals. The transition to the adult stage is marked by the formation of a spherical chamber, a feature that is unique in this species. This terminal chamber is secreted around the thinly calcified trochospiral juvenile shell. At this stage numerous large pores facilitate the migration of the symbionts along the spines. The spherical chamber continues to calcify for a period of 1-7 days (Spero 1988) before spine resorption and gametogenetic (GAM) calcification signal impending gametogenesis. GAM calcification produces 13-28 % of the shell mass over a period of several hours (Bé 1980; Bé et al. 1983; Spero 1986). After gametogenesis the terminal sphere constitutes between 90 to 95 % of the calcite by weight. Frequently the spiral stage is dissolved completely. Reproduction of this species is tuned to the lunar cycle (Bijma et al. 1990).

Globigerina Bulloides

Globigerina bulloides (Fig. 1b) is a non-symbiotic species that is typically associated with temperate to sub-polar water masses but is also characteristic in lower latitudes upwelling environments (Bé and Hutson 1977; Bé and Tolderlund 1971; Naidu and Malmgren 1996a; 1996b). In these regions, *G. bulloides* often dominates the flux to the ocean floor (Sautter and Thunell 1989; Sautter and Sancetta 1992) and is therefore an important source

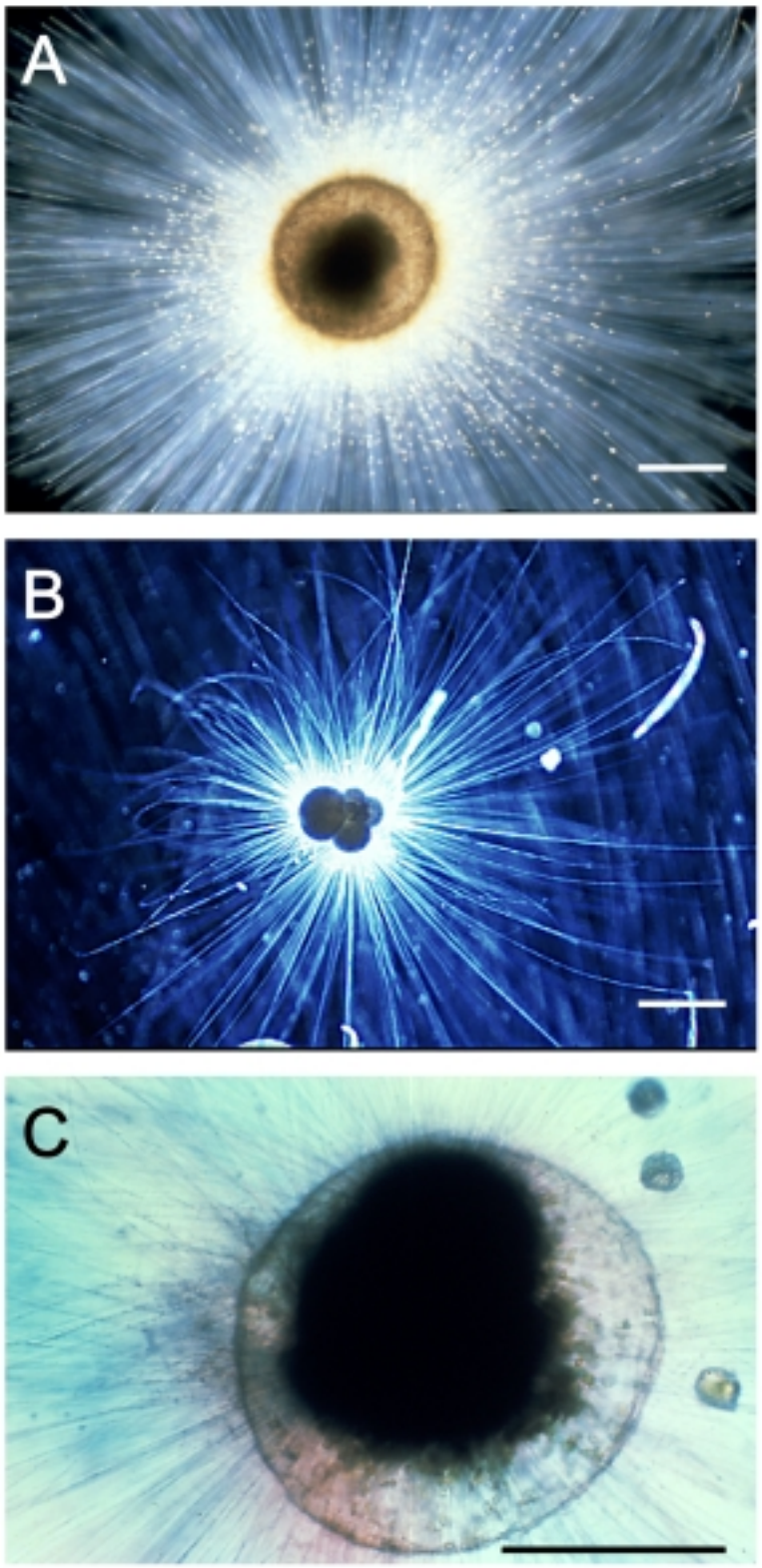

Fig. 1. *Orbulina universa* with symbionts (A; scale bar = 300 μm), *Globigerina bulloides* (B; scale bar = 300 μm), protruding membrane in *O. universa* (C; scale bar = 300 μm).

of geochemical information for paleoceanographic reconstructions (Bard et al. 1987; Kallel et al. 1988; Sautter and Thunell 1991). Contrary to most spinose species, an important part of its diet consists of algae as evidenced by the olive green to brownish coloration of the cytoplasm of freshly collected specimens. Recently, a study on the population dynamics (Schiebel et al. 1997) demonstrated a lunar periodicity of reproduction in this species as well. As evidenced by SEM, gametogenic calcification is much less pronounced than in *O. universa*, but the extent of GAM calcite addition has yet to be quantified.

Culture Protocol

Three sets of experiments were designed to investigate the effect of the carbonate chemistry on shell isotopic fractionation. Culture procedures followed standard protocols (e.g. Bemis et al. 1998; Hemleben et al. 1989). Briefy, *O. universa* and *G. bulloides* were collected by scuba divers from surface waters of the San Pedro Basin, Southern California Bight, U.S.A. and were maintained in laboratory culture at 17 °C and 22 °C (± 0.2 °C). Specimens were grown in 0.8 µm filtered sea water (FSW) whose carbonate chemistry was modified. Sea water ΣCO_2 and total alkalinity (alk_t) were modified via the addition of Na_2CO_3 and/or titration with HCl or NaOH. The pH of the water was determined potentiometrically whereas alk_t and ΣCO_2 were determined by titration and equilibrium calculations respectively. Coulometric determinations of a few sea water samples confirmed the accuracy of the calculated ΣCO_2 values 0(A. Sanyal, pers. comm. 1994). During the experiments, Southern California Bight ambient surface water pH was 8.13 ± 0.02 (at 22 °C) and ambient (alk_t was 2241 ± 19 µeq/kg (n=64). Calculated ΣCO_2 was 2010 ± 18 µmol/kg, while $[CO_3^{2-}]$ was calculated to have varied between 153-184 µmol/kg.

The first two sets of experiments were designed to vary $[CO_3^{2-}]$ at constant alk_t or at constant ΣCO_2. Because $[CO_3^{2-}]$ and pH covary linearly across the relevant pH range in sea water (e.g. 7.9-8.8), these two experiments did not allow us to mechanistically distinguish between pH and $[CO_3^{2-}]$ as a controlling factor. Therefore we designed a third set of experiments at constant pH. The basic difference in the carbonate chemistry between the three experimental groups is that at constant alk_t and constant ΣCO_2, the $[CO_3^{2-}]$ decreases as $[CO_2aq]$ increases whereas at constant pH both carbon species covary (Fig. 2).

The $[CO_3^{2-}]$ of the culture water was modified by:

1) elevating total alkalinity (alk_t) to constant levels of 2842 ± 80 µeq/kg (n=29) and letting pH and ΣCO_2 vary (Fig. 3a, b). We chose to run the experiments at elevated alk_t because removing inorganic carbon quantitatively by acidifying the medium is much more difficult than adding ΣCO_2 or alkalinity. With an alk_t >2800 µeq/kg, $[CO_3^{2-}]$ exceeding 600 µmol/kg could be achieved at ambient ΣCO_2.

2) keeping ΣCO_2 constant at 2032 ± 15 µmol/kg; (n = 15) and letting pH and alk_t vary (Fig. 3c, d and Fig. 4a, b). We chose to work with a slightly elevated experimental ΣCO_2 for the same reason as above.

3) keeping pH constant at 8.15 ± 0.05 and letting ΣCO_2 and alk_t vary. For $[CO_3^{2-}]$ above ambient, we increased ΣCO_2 by adding Na_2CO_3 and subsequently titrated with HCl to bring the pH back to 8.15. For $[CO_3^{2-}]$ below ambient, however, we removed inorganic carbon by acidifying the medium

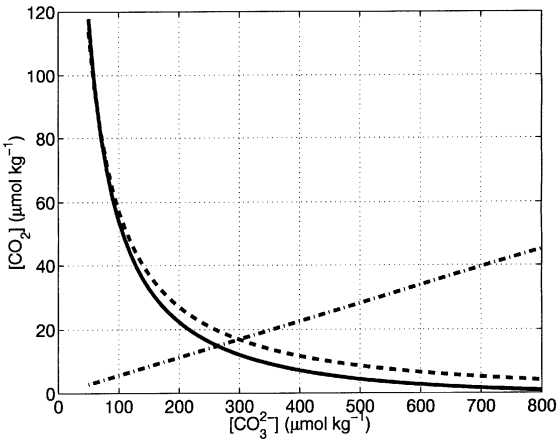

Fig. 2. Relationship between $[CO_3^{2-}]$ and $[CO_2aq]$ at constant alkalinity (broken line), constant ΣCO_2 (solid line) and constant pH (straight broken line).

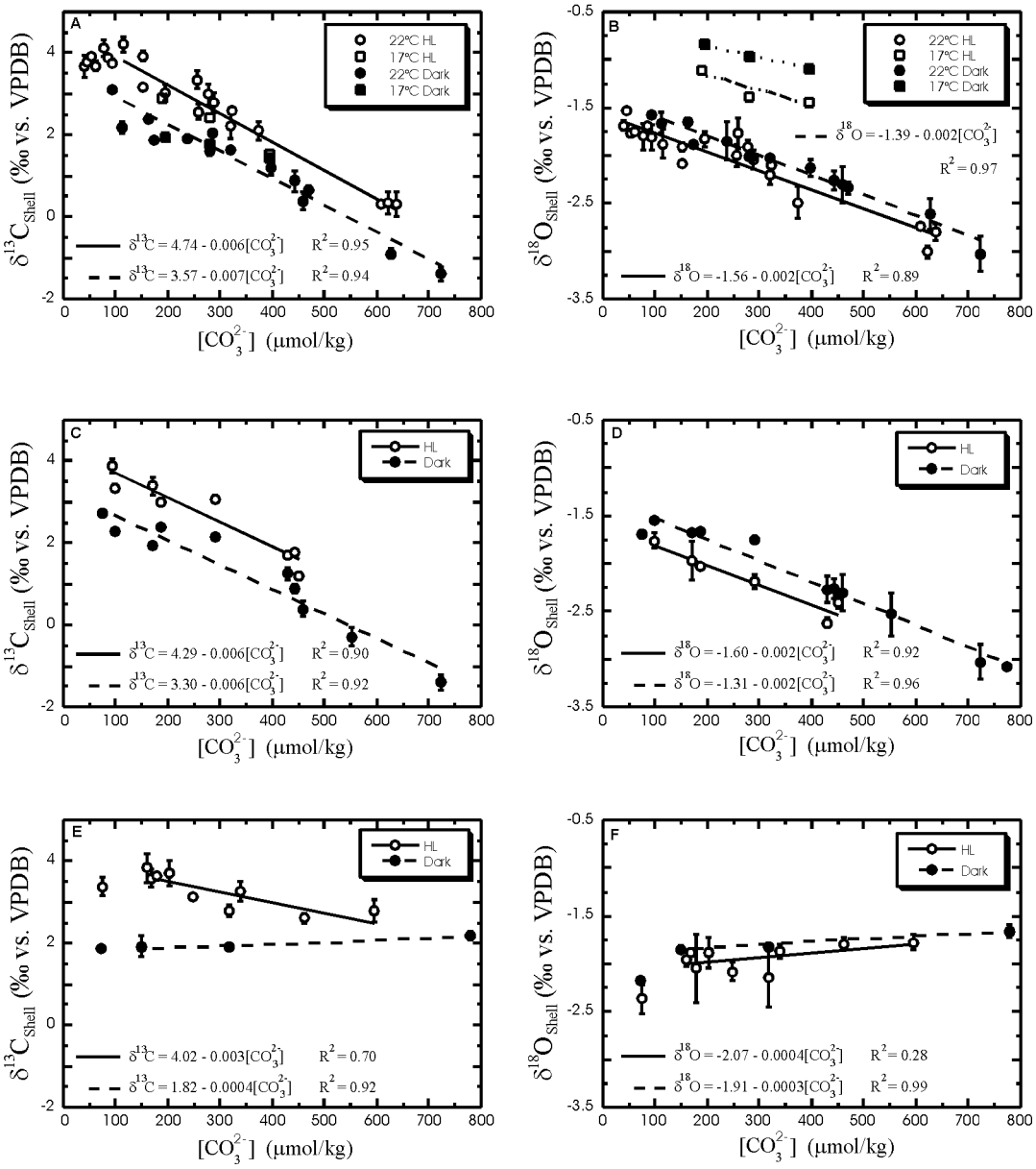

Fig. 3. Effect of CO_3^{2-} ion concentration on the $\delta^{13}C$ and $\delta^{18}O$ values of *Orbulina universa* shell calcite under constant alkalinity (**a** and **b**), constant ΣCO_2 (**c** and **d**) and constant pH (**e** and **f**) conditions. Circles indicate specimens grown at 22 °C and squares indicate specimens grown at 17 °C. Open symbols are mean values of specimens grown under high light (HL), closed symbols are specimens grown in the dark. Plotted data are group mean values ± 1 s.d; most groups are composed of between 3 to 10 individual shell analyses. Lines are linear regressions fitted to the data.

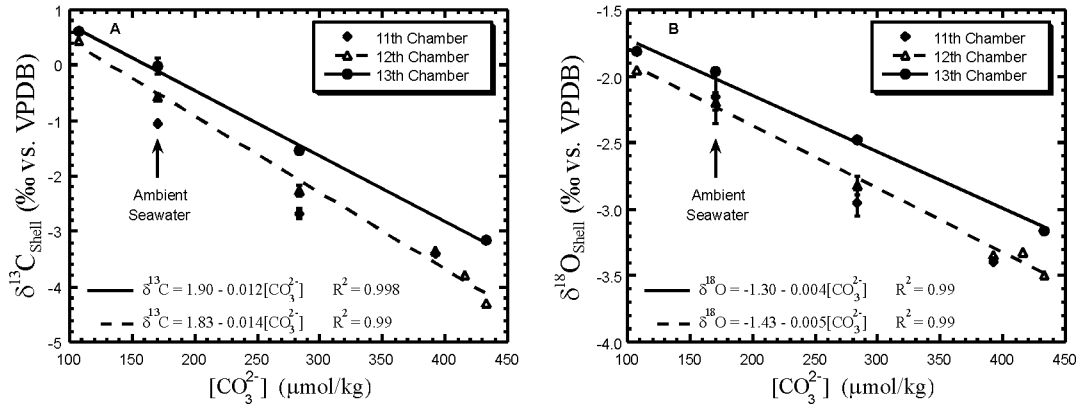

Fig. 4. Effect of CO_3^{2-} ion concentration on the $\delta^{13}C$ **(a)** and $\delta^{18}O$ **(b)** values of *Globigerina bulloides* chamber calcite under constant ΣCO_2 conditions. Chambers from specific positions in the shell whorl are pooled and analysed together to eliminate the effect of ontogenetic isotope variability characteristic of this species (Spero and Lea 1996) Linear regression analyses are plotted for data from chambers 12 and 13, the last two chambers in the shell whorl.

to a calculated intermediate pH (Wolf-Gladrow pers. comm. 1994) at which the ΣCO_2 attained a value that would yield the desired $[CO_3^{2-}]$ at pH 8.15. After reaching the intermediate pH, the headspace was replaced by a N_2 atmosphere to prevent uptake of CO_2 while raising the pH to 8.15 via addition of NaOH.

The specimens were transferred to sealed 125 ml acid-cleaned, air-tight Pyrex™ jars containing the modified culture water and fed an one-day-old artemia nauplius every third day (total time in culture ≈ 6-8 days). For *O. universa*, specimens were maintained in the dark and under non-photoinhibitory P_{max} light levels of 400-700 μE m^{-2} s^{-1} (1 Einstein = 1 mole photons) to quantify the effect of symbiont photosynthesis on this relationship (Spero and Parker 1985). Following gametogenesis, the empty foraminiferal shells were archived for analysis. In *O. universa*, individual spherical chambers were used for isotope analysis. *Globigerina bulloides* chambers secreted during the experiments were amputated from the shell whorl and pooled according to chamber position (Spero and Lea 1996). The samples were roasted at 375 °C *in vacuo* and analyzed with a Fisons Optima isotope ratio mass spectrometer using an Isocarb common acid bath autocarbonate system at 90 °C.

To confirm the stability of the carbonate chemistry and isotopic composition of the culture water throughout each experiment, water samples were collected at the start and end of each experiment to analyse alkalinity, ΣCO_2, $\delta^{13}C_{\Sigma CO2}$ and $\delta^{18}O_{water}$.

Cytoplasmic Inorganic Carbon Pool

To explain the stable carbon isotopic signature in a variety of calcifying organisms, a so called internal inorganic carbon pool has been proposed (e.g. Erez 1978a; Erez 1978b; Goreau 1963; Goreau 1977; Kuile et al. 1989; Weber and Woodhead 1970). To investigate the presence of such a cytoplasmic inorganic carbon pool in *O. universa*, pulse-chase experiments were designed. Freshly collected *O. universa* were fed and placed in ^{13}C-spiked solution (after a rinse through a ^{13}C-spiked medium). The specimens were monitored at regular intervals to catch the moment of membrane protrusion (Fig. 1c) At this stage, the specimens were rinsed three times in normal FSW, transferred to unspiked culture water and allowed to calcify the spherical chamber (see also Lea et al. 1995). Specimens were kept in the dark or at P_{max} light levels and fed every third day. After gametogenesis, the specimens were archived and processed as described above.

A spike stock-solution was prepared by dissolving 7 mg of 99.1 % $Na_2{}^{13}CO_3$ in 100 ml deionized water. From this stock-solution 35 ml were added to 1 l FSW (2028 µMol ΣCO_2; $\delta^{13}C_{\Sigma CO2} \approx 2$ ‰). Initial and final samples of the ^{13}C-spiked culture medium were measured, indicating that the average spike was 77.8 ‰ ± 5.2 ‰ (n=11). The pulse- and chase-solution for each experiment were taken from the same batch of modified sea water and therefore had identical carbonate chemistry. The $[CO_3^{2-}]$ varied between 153 and 517 µmol/kg at an average constant alkalinity of 2563 ± 371 µeq/kg.

Results

Water samples collected at the start and end of each experiment showed that the carbonate chemistry (as determined by pH, alk_t and equilibrium calculations) remained constant throughout the experiments. The isotope chemistry ($\delta^{13}C_{SCO2}$ and $\delta^{18}O_{water}$) of the culture medium remained fairly constant as well. Analyses show that $\delta^{18}O_{water}$ was constant at -0.23 ± 0.05 ‰ (vs. VSMOW) (n=100). The $\delta^{13}C_{SCO2}$ depended on the amount of Na_2CO_3 that was added to modify the carbonate chemistry in each experiment. Analysis of initial and final water show that $\delta^{13}C_{SCO2}$ varied on average 0.16 ± 0.10 ‰ (n=30).

All foraminiferal $\delta^{13}C$ data have been standardized to a $\delta^{13}C_{\Sigma CO2}$ = 2.00 ‰ (ambient sea water $\delta^{13}C_{\Sigma CO2}$ = 1.90 ± 0.08 ‰, n=18) by adding or subtracting the difference between 2.00 ‰ and the measured experimental water $\delta^{13}C_{\Sigma CO2}$ to each shell value. Here $\delta^{18}O$ (and similar for $\delta^{13}C$) = $[(^{18}O/^{16}O_{sample}/\ ^{18}O/^{16}O_{standard}) - 1] \times 1000$. All isotope values are relative to VPDB unless noted. To convert $\delta^{18}O_w$ values from the VSMOW to VPDB scale, the most recent correction of -0.27 ‰ (Hut 1987) was used.

Constant Total Alkalinity Experiment

Results demonstrate that *O. universa* shell $\delta^{13}C$ and $\delta^{18}O$ values decrease approximately 3.9 ‰ and 1.5 ‰ respectively as carbonate increases from 41 to 642 µmol/kg (pH increases from 7.38 to 8.83) (Fig. 3a, b). Shell $\delta^{13}C$ values do not increase further when sea water $[CO_3^{2-}]$ drops below ca. 100 µmol/kg. Comparison of foraminifers grown under high light (HL = maximum symbiont photosynthesis) with specimens maintained in the dark (no symbiont photosynthetic activity) indicates that HL shells are enriched in ^{13}C by 1.1 ‰ and depleted in ^{18}O by 0.3 ‰ relative to dark specimens. These offsets are seen across the full range of $[CO_3^{2-}]$. Although the effect of symbiont photosynthesis on shell $\delta^{13}C$ and $\delta^{18}O$ was documented previously (Spero and DeNiro 1987; Spero and Lea 1996; Spero and Williams 1988), the fact that the $\delta^{13}C/[CO_3^{2-}]$ and $\delta^{18}O/[CO_3^{2-}]$ slopes from HL and dark experiments are ca. 0.0065 and 0.002 ‰/µmol kg^{-1} respectively (Table 1) indicates that symbiont photosynthesis does not affect the stable isotope:carbonate ion relationship.

Six experiments were carried out at a 5°C lower culture temperature. Although, the temperature decrease does not seem to significantly affect the $\delta^{13}C/[CO_3^{2-}]$ nor the $\delta^{18}O/[CO_3^{2-}]$ relationship, additional experiments are needed to further bolster this conclusion.

Constant Total Inorganic Carbon Experiment

In a second series of experiments, we manipulated the carbonate concentration between 75 - 774 µmol/kg (pH increase from 7.87 to 8.97) by varying alk_t and maintaining ΣCO_2 at ambient values (Fig 3c, d). *Orbulina universa* shell $\delta^{13}C$ and $\delta^{18}O$ decreased as $[CO_3^{2-}]$ increased, with similar slopes as under constant alk_t: 0.006 and 0.002 ‰/µmol kg^{-1} respectively (Table 1). Again, comparison of foraminifers grown under HL with specimens maintained in the dark (no symbiont photosynthetic activity) indicates that HL shells are enriched in ^{13}C by 1.1 ‰ and depleted in ^{18}O by 0.4 ‰ relative to dark specimens and that these offsets are constant across the full range of $[CO_3^{2-}]$.

We also cultured the non-symbiotic species, *G. bulloides*, across a $[CO_3^{2-}]$ range of 103-436 µmol/kg at constant ΣCO_2. Because *G. bulloides* displays a large chamber-to-chamber ontogenetic effect for both $\delta^{13}C$ and $\delta^{18}O$ (Spero and Lea 1996), we amputated chambers secreted during the

experiments and pooled them according to their discrete positions in the shell whorl for stable isotope measurement (Fig. 4a, b). Regression analyses on data from the 11th, 12th and 13th chamber groups yield similar $\delta^{13}C/[CO_3^{2-}]$ and $\delta^{18}O/[CO_3^{2-}]$ slopes demonstrating that ontogenetic effects have little or no affect on this relationship. However, the average *G. bulloides* $\delta^{13}C/[CO_3^{2-}]$ and $\delta^{18}O/[CO_3^{2-}]$ slopes of 0.013 and 0.0045 ‰/µmol kg^{-1} are twice that of *O. universa* (Table 1).

Constant pH Experiment

Results demonstrate that shell $\delta^{13}C$ and $\delta^{18}O$ values of *O. universa* grown in the dark are approximately constant (2.0 ‰ and -1.75 ‰ respectively) as carbonate increases from ambient to 780 µmol $[CO_3^{2-}]$/kg (ΣCO_2 increases from 748 to 9,273 µmol/kg; alk_t increases from 875 to 10,073 µeq/kg) (Fig. 3e, f). If anything, there is an insignificant but distinct trend towards slightly higher values for $\delta^{13}C$ and $\delta^{18}O$ with increasing $[CO_3^{2-}]$ in the dark. Comparison of foraminifers grown under HL (maximum symbiont photosynthesis) with specimens maintained in the dark indicates that HL shells are enriched in ^{13}C by ca. 1.5 ‰ at ambient $[CO_3^{2-}]$, but that this enrichment decreases as $[CO_3^{2-}]$ increases. Because this offset is not constant across the full range of $[CO_3^{2-}]$, symbiont photosynthesis does have an effect on the $\delta^{13}C/[CO_3^{2-}]$ relationship under constant pH. On the contrary, the $\delta^{18}O$ values in the light are slightly depleted compared to those in the dark and the offset (≈ 0.1 ‰) is more or less constant across the full range of $[CO_3^{2-}]$.

Inorganic Carbon Pool (Pulse-Chase) Experiment

After a mean incubation time of two days in the "pulse" solution ($\delta^{13}C_{\Sigma CO2}$ = 77.8 ‰ ± 5.2 ‰), the specimens were transferred, upon membrane protrusion, to a "chase" solution with the same carbon-

Species	Experiment		Regression Slope	
			$\frac{\delta^{13}C}{[CO_3^{2-}]}$	$\frac{\delta^{18}O}{[CO_3^{2-}]}$
Orbulina universa	Constant Alkalinity	High Light	0.006	0.002
		Dark	0.007	0.002
	Constant ΣCO_2	High Light	0.006	0.002
		High Light*	0.006	0.0012
		Dark	0.006	0.002
Globigerina bulloides	Constant ΣCO_2	12th Chamber	0.014	0.005
		13th Chamber	0.012	0.004
		11th Chamber*	0.013	0.005
		12th Chamber*	0.012	0.004
		13th Chamber*	0.013	0.004

* Data from a feeding experiment not described here

Table 1. Experiment regression slopes.

ate chemistry as the "pulse" solution, fed as usual and left to calcify the adult chamber and undergo gametogenesis. It can be calculated that the small amount of the "pulse" solution that is enclosed by the membrane and inevitably transferred to the chase culture medium does not affect the shell $\delta^{13}C$ even if all of this "enclosed" carbon is used for calcification (cf. Lea et al. 1995). The $\delta^{13}C$ values of the spiked specimens were slightly higher than those of the control group (Fig. 5a-d) but mass balance calculations demonstrate that less than 1 % of the carbon used for calcification originates from the spike and hence that, if present at all, the inorganic carbon pool is insignificant.

Discussion

Because the sea water carbonate chemistry system was modified in fundamentally different ways (varying proportions of ΣCO_2 and alk_t) yet produced indistinguishable regression slopes, the observed stable isotope:carbonate ion relationship is real and not an artifact of our experiments. In addition, identical $\delta^{13}C$ and $\delta^{18}O$ values of pre- and post-gametogenic shells (Spero et al. 1997) demonstrate that the relationships are a function of calcification during normal growth and not due to inorganic precipitation on the surface of the empty post-gametogenic shell.

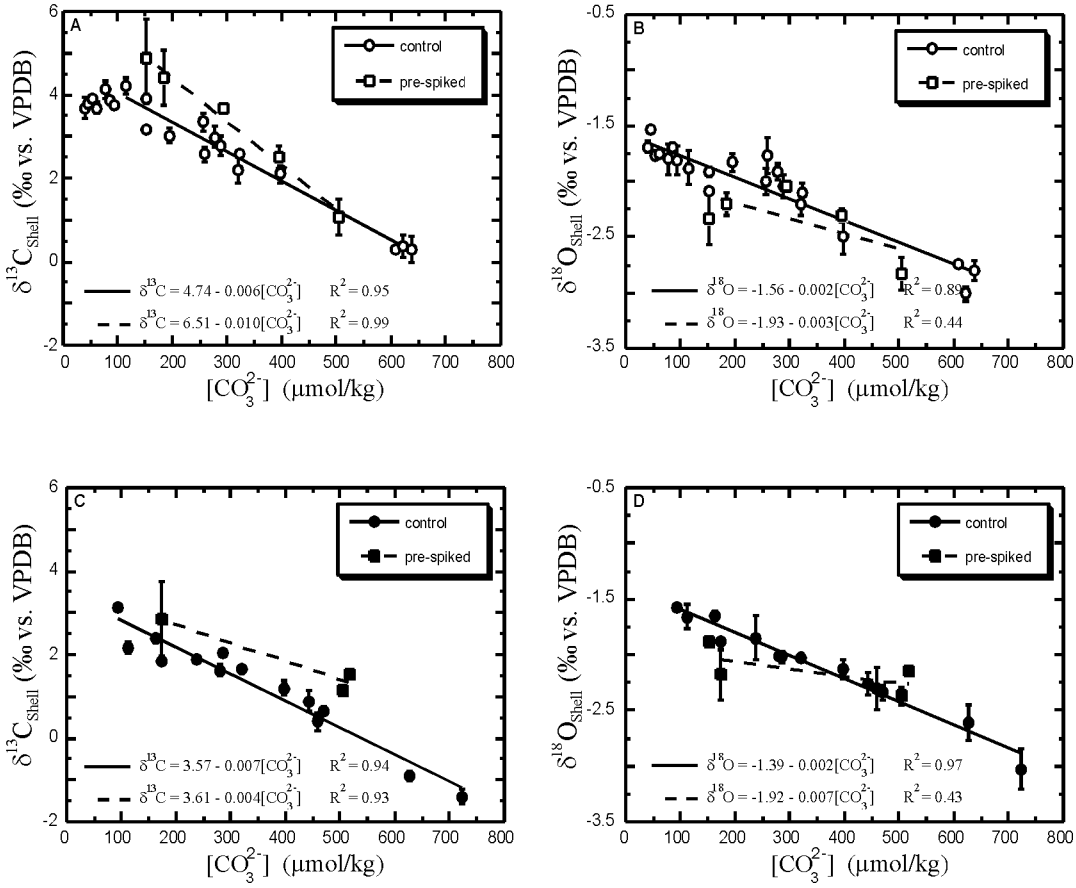

Fig. 5. Effect of pre-culture labeling ($\delta^{13}C_{\Sigma CO2}$ = 77.8 ‰) on the $\delta^{13}C$ and $\delta^{18}O$ values (under constant alkalinity conditions) as a function of CO_3^{2-} ion concentration (squares). For comparison, the data of the constant alkalinity experiment (Fig. 2 a,b) are also plotted (circles). Open symbols **(a, b)** are mean values of specimens grown under high light, (HL); closed symbols **(c, d)** are specimens grown in the dark. Plotted data are group mean values ± 1 s.d. The lines are linear regressions fitted to the data.

The experiments carried out with the symbiont barren *G. bulloides* as well as the dark experiments with *O. universa* clearly demonstrate that whatever controls the stable isotope:carbonate chemistry relationship is independent of symbiont activity. Experiments carried out at a 5°C lower temperature further suggest that this relationship is also independent of temperature. In addition, an unexpected stable oxygen isotope:[CO_3^{2-}] relationship (also independent of symbiont activity and temperature) was found that cannot be explained in terms of known "vital effects". These observations force us to reassess previously proposed fractionation mechanisms.

In the following sections we will first discuss inorganic equilibrium and kinetic fractionation mechanisms. We will then discuss the influence of respiration and photosynthesis and finally propose a combination of mechanisms that control the isotopic composition of symbiont barren and symbiont bearing foraminifers.

Equilibrium and Kinetic Fractionation

Mass balance calculations (Mook et al. 1974; Zhang et al. 1995) show that the $\delta^{13}C$:carbonate chemistry relationship documented here cannot be explained in terms of equilibrium fractionation, i.e. by a simple redistribution of isotopes between the dissolved carbon species (Fig. 6). The influence of temperature (17 vs. 22 °C) on the isotopic redistribution between the carbon species is only discernible in the $\delta^{13}C$ composition of CO_2. The $\delta^{18}O$ signal of the carbon species is insensitive to changes in the carbonate chemistry because the water reservoir is huge compared to the ΣCO_2 reservoir.

With regard to kinetic fractionation, the early inorganic precipitation experiments of McCrea (1950) are of particular interest. He demonstrated that the $^{18}O/^{16}O$ ratio of rapidly precipitated $CaCO_3$ decreases with increasing percentage CO_3^{2-} in solution. McCrea proposed that the oxygen isotope equilibrium fractionation between the sum of the dissolved carbonate species and water is linearly related to the equilibrium between the carbonate species, a conclusion that was, more than 40 years later, confirmed by Usdowski and Hoefs (1993). Comparison of our experimental *O. universa* data with those of McCrea show remarkable similarity in the slopes of the relationships (Fig. 7). We note that the larger *G. bulloides* $\delta^{18}O$:[CO_3^{2-}] relationship demonstrates that additional fractionation mechanisms are operative. McCrea proposed that the $\delta^{18}O$:[CO_3^{2-}] relationship for inorganic precipitates could be a function of calcification rate. This conclusion is not supported by our observations because: 1) Above ambient [CO_3^{2-}], the average shell weight, in both the dark and HL, is fairly constant (albeit variable) with increasing [CO_3^{2-}]. Because the life span of *O. universa* was similar between and within treatments, the average calcification rate must have been relatively constant in the [CO_3^{2-}] range above ambient as well. On the contrary, below ambient [CO_3^{2-}], the calcification rates in the dark and in HL decrease significantly with decreasing [CO_3^{2-}] (Fig. 8). Thus, if it is assumed that changes in the calcification rate control the $\delta^{18}O$ fractionation we would expect a clear break in the shell $\delta^{18}O$:[CO_3^{2-}] relationship at ambient [CO_3^{2-}]. On the contrary, the shell $\delta^{18}O$:[CO_3^{2-}] relationship decreases linear across the full [CO_3^{2-}] range. 2) The difference in shell weight, and by extension the difference in calcification rate, between the HL and dark experiments (cf. Lea et al. 1995) is larger than the difference in shell weight

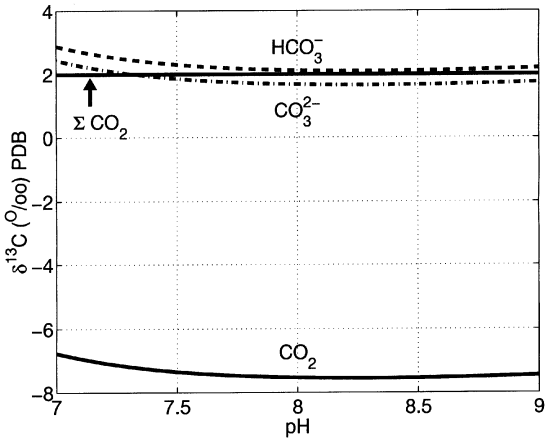

Fig. 6. Mass balance calculations (Mook et al. 1974; Zhang et al. 1995) show the redistribution of isotopes between the dissolved carbon species as a function of pH.

between ambient and high [CO_3^{2-}] (Fig. 8). Consequently, if the fractionation is controlled by the calcification rate, the light/dark effect on $\delta^{18}O$ should be larger than the [CO_3^{2-}] effect. On the contrary, the light/dark shift in $\delta^{18}O$ is on the average -0.35 ‰ for *O. universa* and constant across the full range of the experimental [CO_3^{2-}] while the depletion is approximately 1.5 ‰ over a [CO_3^{2-}] range of ca. 600 and 700 µmol/kg at constant alk_t and constant ΣCO_2, respectively. Shells of *G. bulloides* are depleted by 1.4 ‰ across a [CO_3^{2-}] range of only 333 µmol/kg (at constant ΣCO_2). Again, this suggests that the calcification rate does not control ^{18}O incorporation. 3) Finally, the final shell weight of *O. universa*, ranging between 10 to 70 µg, is very variable between and within treatments (Fig. 8). Thus, if the calcification rate is the controlling factor, the correlation between the oxygen isotopic composition of the shell and the percentage carbonate ion in solution (Fig. 7) should be very low. At this stage we therefore have to conclude that the $\delta^{18}O$:[CO_3^{2-}] relationship for inorganic and biological precipitates is probably not a function of calcification rate as proposed by McCrea. However, because we find it difficult to believe that the rate at which a calcifying organism accretes $CaCO_3$ is not a fundamental factor for isotope fractionation, additional experiments will have to be carried out to confirm this hypothesis. It should also be noted that although the similarity in life span of *O. universa* between and within treatments can be used to roughly quantify calcification rate (because spherical chamber formation is a continuous process) it cannot be applied to *G. bulloides* because chambers are formed discretely.

More importantly, the similarity between our investigation and the inorganic precipitate study of McCrea implies a kinetic mechanism which would affect all calcifying organisms and inorganic precipitates. Evidence for a common kinetic fractionation mechanism exists in corals and other invertebrate groups. For instance, McConnaughey (1989b) demonstrated that the carbon and oxygen isotopic composition of symbiotic and non-symbiotic coral skeletons can vary significantly across skeletal surfaces that were presumably secreted synchronously. In the non-photosynthetic species, *Tubastrea*, the values covary with a posi-

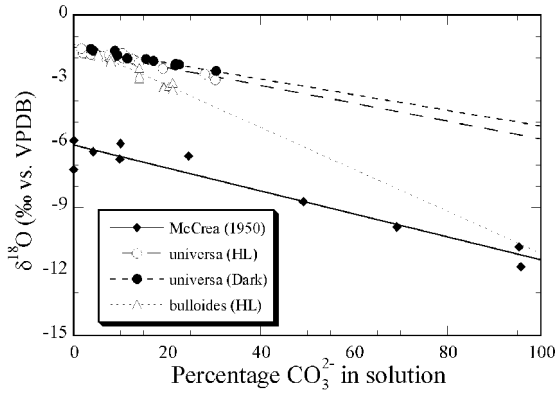

Fig. 7. Comparison of high light (open circles) and dark (closed circles) *O. universa* $\delta^{18}O$ data from the constant alkalinity experiment and high light *G. bulloides* $\delta^{18}O$ data (open triangle) from the constant ΣCO_2 experiment with inorganic precipitate results from McCrea (1950). Oxygen isotope data are plotted vs. percentage CO_3^{2-} in solution where: % CO_3^{2-} = [CO_3^{2-}] / ([CO_3^{2-}] + [HCO_3^-]). Note that the slopes of the *O. universa* data are indistinguishable from that of the McCrea data set but that the slope of the *G. bulloides* data is much steeper.

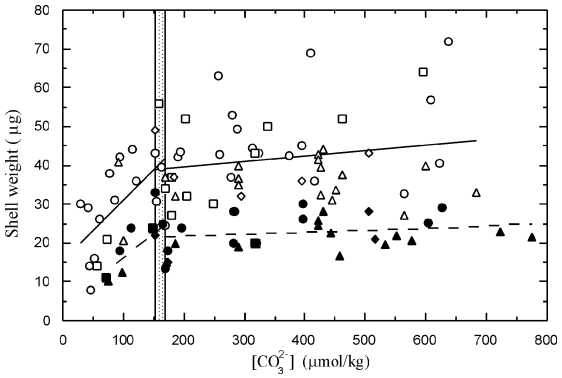

Fig. 8. Shell weights of *O. universa* plotted as a function of CO_3^{2-} ion concentration. Chamber mass data for *G. bulloides* were inconclusive and are therefore not shown. The shaded area represents the range of ambient [CO_3^{2-}]. Circles, triangles and squares represent the constant alkalinity, ΣCO_2 and pH experiment, respectively. Diamonds indicate the pulse-chase experiments. Open symbols are mean values of specimens grown under high light (HL), closed symbols are specimens grown in the dark. Linear regressions were fitted to data below and above ambient [CO_3^{2-}]; HL data (solid line) and dark data (broken line).

tive $\delta^{18}O/\delta^{13}C$ slope of 0.29. The foraminiferal $\delta^{18}O/\delta^{13}C$ slopes derived from the experimental data are virtually identical to *Tubastrea*, with values ranging between 0.29 and 0.33 (Fig. 9). Other ahermatypic corals, calcareous algae and invertebrates such as cidaroid urchins show similar oxygen and carbon isotope covariance (McConnaughey 1989b; Wefer and Berger 1991) although the $\delta^{18}O/\delta^{13}C$ slopes can differ from the experimental range reported here. For instance, in symbiont-bearing organisms such as the coral *Pavona*, the slope can approach unity or even change sign due to the additional ^{13}C-enriching effect of symbiont photosynthesis (McConnaughey 1989b). Aside from organisms that display a large symbiont effect, the slope relationship similarities among different protozoans, invertebrate groups and some calcifying algae suggest that a common kinetic mechanism is responsible for the observed covariance. Moreover, the linearity between $\delta^{18}O$ and $\delta^{13}C$ suggests that whatever controls the fractionation of oxygen also affects carbon.

McConnaughey (1989a) has proposed that the fractionation observed in *Tubastrea* occurs during CO_2 hydration and hydroxylation to form HCO_3^-.

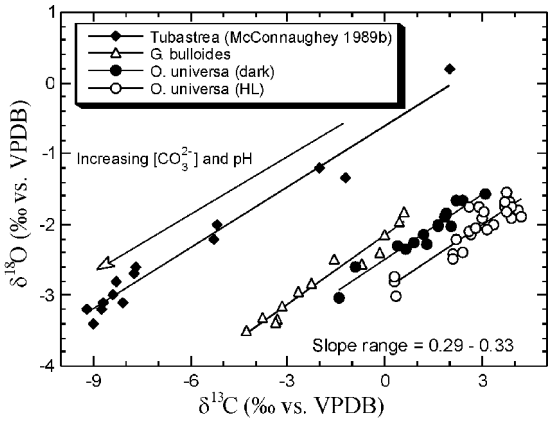

Fig. 9. Comparision of foraminiferal carbon and oxygen isotope data from all experiments with data from the non-symbiotic coral, *Tubastrea* spp (McConnaughey 1989b). The similarity of the positive covariance between coral (aragonite) and foraminiferal (calcite) stable isotopes suggest that similar mechanisms are responsible for the observed variations. In this plot, $[CO_3^{2-}]$ and pH increase towards the origin.

The rates of hydration and hydroxylation are pH dependent and are the rate-limiting steps responsible for the kinetic discrimination against the heavier ^{18}O and ^{13}C isotopes in the calcifying microenvironment adjacent to the skeleton. McConnaughey further argues that CO_2 hydration and hydroxylation reactions may exhibit different kinetic isotope effects and that the balance between these two reactions changes with pH. Subsequent isotopic equilibration between HCO_3^- and CO_3^{2-} is effectively instantaneous because the protonation of CO_3^{2-} and the deprotonation of HCO_3^- are extremely fast. With regard to $\delta^{18}O$, McConnaughey's prediction is in agreement with results from inorganic precipitation experiments (Usdowski et al. 1991).

pH or Carbonate Ion Control

Our experiments allow us to mechanistically distinguish between pH and $[CO_3^{2-}]$ as controlling factors for the observed fractionation. The fact that under constant pH, $\delta^{13}C$ and $\delta^{18}O$ of shells kept in the dark do not vary with increasing $[CO_3^{2-}]$ suggest that pH, as proposed by McConnaughey (1989a), controls the stable isotope fractionation and not $[CO_3^{2-}]$. This conclusion is supported by experiments that were recently carried out with *G. sacculifer* at constant pH (Bijma, Spero and Lea unpubl. results). Fig. 10a and b demonstrate that, at constant alkalinity, below pH ≈ 8 the fractionation is approximately constant (the $C\Sigma CO_2$ experiments suggest a similar trend). Apparently, the partitioning of the hydration and hydroxylation does not change very much in that pH range. One could argue that, although insignificant, the dark constant pH experiments show a trend towards higher values for both $\delta^{13}C$ and $\delta^{18}O$ at higher $[CO_3^{2-}]$. At present we have no explanation for this observation. It can neither be justified by a redistribution of isotopes between the dissolved carbon species as pH is more or less constant (Fig. 11c) nor by a change in the relative proportion of light metabolic CO_2 incorporation (because the $\delta^{18}O$ difference between respired and bulk CO_2 is negligible as will be demonstrated later).

An intriguing question is why the shell stable isotope composition responds linear to $[CO_3^{2-}]$

(Figs. 3,4) but not to pH (Fig. 10)? Intuitively one would conclude that kinetic fractionation is apparently controlled by the $[CO_3^{2-}]$. However, in a non-equilibrium situation where the kinetic fractionation is dependent on the balance between CO_2 hydration and hydroxylation as suggested by McConnaughey (cf. Fig. 6 in McConnaughey 1989a) and both reactions exhibit different but constant kinetic isotope effects, the pH-$\delta^{13}C_{\Sigma CO2}$ (cf. Fig. 6) and the pH-$\delta^{18}O_{\Sigma CO2}$ relationships are not constant but apparently decrease with increasing pH (Fig. 10a-d). Because the pH/$[CO_3^{2-}]$ relationship in the constant alkalinity and the constant ΣCO_2 experiments is also non-linear (Fig. 11a, b), the $[CO_3^{2-}]$:stable isotope relationships in these experiments turn out to be linear. On the other hand, as argued before, we believe that the calcification rate (for instance, the time available for isotopic equilibration between CO_3^{2-} and solid $CaCO_3$ before subsequent layers prevent isotopic exchange) should play an important role and is likely to depend on the saturation state (Ω) and hence on the $[CO_3^{2-}]$. Independent of which factor ultimately controls the relationship between the carbonate chemistry and isotopic fractionation, the dependence may be expressed as a function of pH or $[CO_3^{2-}]$. For obvious reasons we prefer the linear relationship with $[CO_3^{2-}]$. It should also be noted, that in the real ocean $[CO_3^{2-}]$ and pH covary linearly across the relevant pH range.

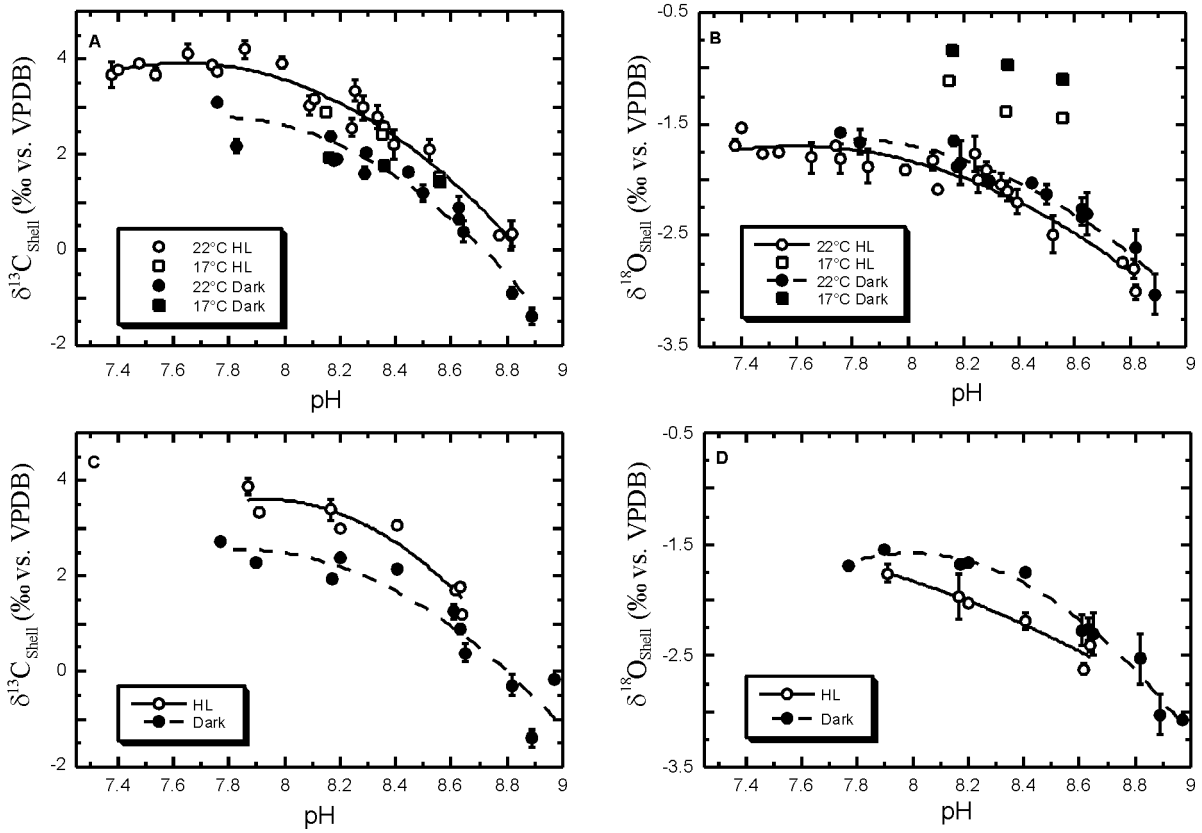

Fig. 10. Effect of pH on the $\delta^{13}C$ and $\delta^{18}O$ values of *Orbulina universa* shell calcite under constant alkalinity **(a** and **b)** and constant ΣCO_2 **(c** and **d)**. Circles indicate specimens grown at 22°C and squares indicate specimens grown at 17°C. Open symbols are mean values of specimens grown under high light (HL), closed symbols are specimens grown in the dark. Plotted data are group mean values ± 1 s.d. Lines are linear regressions fitted to the data.

Impact of Respired CO_2

Because isotopes are conserved in metabolism, the extent of ^{13}C depletion of respired CO_2 must reflect the $\delta^{13}C$ of the carbon source and the carbon budget of the consumer. Thus, the $\delta^{13}C$ of respired CO_2 depends on the $\delta^{13}C$ value of the food source and the $\delta^{13}C_{org}$ of the foraminifer. The following mass balance calculation may be considered:

$$\delta^{13}C_{source} = [r \bullet \delta^{13}C_{foram}] + [(1-r) \bullet \delta^{13}C_{resp}]$$

where r is the fraction of carbon retained by the foraminifer. Rearranged:

$$\delta^{13}C_{resp} = (\delta^{13}C_{source} - [r \bullet \delta^{13}C_{foram}])/(1-r)$$

For symbiont bearing species it was demonstrated that a substantial fraction (denoted s) of the host's carbon is derived from the symbionts, probably translocated during the night when the symbionts are retracted in the shell (Bemis pers. comm.). Thus for *O. universa* the equation must be expanded:

$$\delta^{13}C_{resp} = ([s \bullet \delta^{13}C_{symbiont}] + [1 - s \bullet \delta^{13}C_{food}] - [r \bullet \delta^{13}C_{foram}])/(1-r)$$

$\delta^{13}C$ of the artemia, the host and the symbionts is a function of their biochemical composition, especially of the lipid content. Corrected for the carapace, that is discarded after digestion, the $\delta^{13}C$ value a Great Salt Lake artemia nauplii (GSL) is -15 ‰. The $\delta^{13}C_{org}$ of *O. universa* and *G. bulloides* fed GSL artemia is -17.4 ‰ and -18.5 ‰ respectively (Uhle unpubl. results). Based on compound specific isotope analysis (Table 2) and the assumption that lipids are 5 ‰ depleted compared to the average $\delta^{13}C$ of symbiont organic matter, a $\delta^{13}C_{symbiont}$ of -24 ‰ can be estimated (Schouten pers. comm.). If we further assume that 56 % of the carbon taken up by *O. universa* originates from the GSL-artemia and that the rest is derived from the symbionts (Bemis pers comm) and that only one tenth of the carbon taken up is retained, the $\delta^{13}C$ of the respired CO_2 is -19.1 ‰ and -14.6 ‰ for *O. universa* and *G. bulloides* respectively (Table 3). Bemis (unpubl data) determined that the $\delta^{13}C_{org}$ of *O. universa* fed artemia nauplii of a San Francisco Bay (SFB) strain was -18.6 ‰, while the artemia were on the average -20.4 ‰ (corrected for the carapace). The $\delta^{13}C$ of the respired CO_2 under these conditions is -22.4 ‰ (Table 3). Unfortunately, we have no data for *G. bulloides* fed SFB artemia.

Respiring organisms consume $^{16}O^{16}O$ 10 to 20 ‰ more rapidly than $^{18}O^{16}O$. With respect to

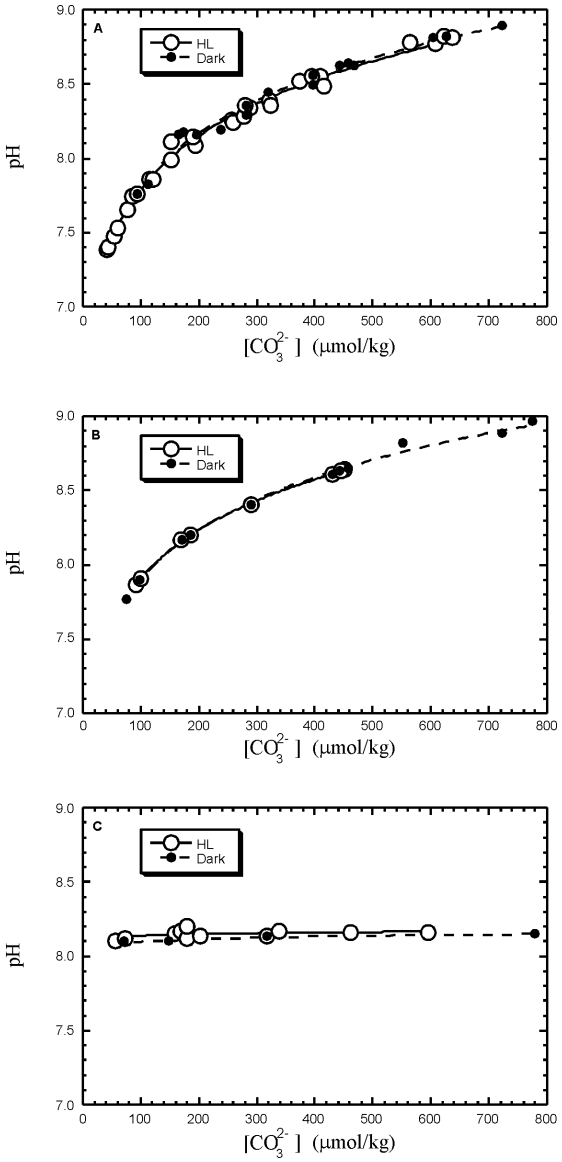

Fig. 11. Relationship between $[CO_3^{2-}]$ and pH at constant alkalinity **(a)**, constant ΣCO_2 **(b)** and, constant pH **(c)**.

the metabolic fractionation during respiration a fractionation factor of 1.016 has been proposed for oxygen (Lane and Dole 1956). Others (Kiddon et al. 1993), measured a respiratory isotope effect between 14 ‰ to 26 ‰ for seven representative unicellular marine organisms and estimate that the average fractionation of the dominant marine respirers is about 20 ± 3 ‰. A generalized respiration equation is given by:

$$CH_2O + O_2 \rightarrow CO_2 + H_2O$$

"free sterols"	$\delta^{13}C$
cholest-5,22-dienol	-29.5 ‰ ± 0.2 ‰
cholesterol (=cholest-5-enol)	-28.7 ‰ ± 0.2 ‰
ergosta-5,22-dienol	-29.1 ‰ ± 0.1 ‰
ergosta-5,24(28)-dienol	(-24.9 ‰ ± 0.1 ‰)
average	ca. -29 ‰

Table 2. Stable carbon isotope values of dominant free sterols. Ergosta-5,24(28)-dienol was not used for the determination of the average $\delta^{13}C$. We believe that the much heavier isotope signature of this compound originates from heterotrophic "swarmers".

artemia strain	$\delta^{13}C$resp (‰)	
	O. universa	*G. bulloides*
GSL (-15 ‰)	-19.1	-14.6
SFB (-20.4 ‰)	-22.4	n.a.

Table 3. Calculated $\delta^{13}C$ of respired CO_2 for a symbiont bearing and a symbiont barren species fed two different strains of artemia (GSL = Great Salt Lake; SFB = San Francisco Bay).

If we assume a fractionation of 20 ‰ during respiration and that the $\delta^{18}O$ of O_2 is -0.45 ‰ vs. VSMOW (equivalent to H_2O) and if we further assume that the $\delta^{18}O$ of the combined host/symbiont organic matter is 28 ‰ (Swart 1983) and that CO_2 and H_2O maintain their characteristic fractionation of 1.0412 at 25 °C (Friedman and O'Neil 1977), respiration may produce a pool of ^{18}O-depleted CO_2 and cell H_2O. However, due to the large cell water reservoir and the large water fluxes across the membrane (according to McConnaughey (1989a) 6 orders of magnitude larger than photosynthetic or respiration fluxes) the $\delta^{18}O$ of the cell water quickly returns to -0.45 ‰. Thus, although respired CO_2 can be 13 ‰ depleted compared to $\delta^{18}O$ of bulk CO_2, the fast isotope exchange with cell water may completely cancel this offset. However, once converted to CO_3^{2-} a part of the signal may be preserved due to the slow exchange of oxygen isotopes between dissolved CO_3^{2-} and H_2O (McConnaughey 1989a).

In summary, incorporation of respired CO_2 can significantly lower shell $\delta^{13}C$ but does probably not affect $\delta^{18}O$. For example, Spero and Lea (1996) demonstrated that *G. bulloides*, when fed SFB artemia is ca. 0.5 ‰ depleted in ^{13}C compared to specimens fed GSL artemia but that shell $\delta^{18}O$ was unaffected. Thus, incorporation of respired CO_2 lowers shell $\delta^{13}C$ but the effect is relatively small compared to the ca. 3 times larger photosynthetic effect that has been determined for *O. universa* and *G. sacculifer*. However, the calculations by Spero and Lea (1996) are based on the isotopic difference of the food source. In the following we will use the calculated isotopic difference in the respired CO_2 (Table 3). If all respired CO_2 would be incorporated into the shell (100 % efficiency), the shell $\delta^{13}C$ difference of specimens fed SFB or GSL artemia should be 3.3 ‰, i.e. reflect the full difference in the carbon isotopic composition of respired CO_2. Since the difference in shell $\delta^{13}C$ of *G. sacculifer* fed SFB and GSL artemia, is on the average only 0.55 ‰ (Bijma, Spero and Lea unpubl. results) it can be calculated that the impact of respiration on the shell isotopic value is ca. 17 %. Consequently, with an efficiency of 17 %, respiration can lower the shell isotopic

composition by 3.2 to 3.8 ‰, respectively, when fed GSL and SFB artemia.

Symbiont Effect

Our experimental results have separated the influence of symbiont photosynthesis from the influence of the carbonate chemistry on the shell isotopic composition calcification. The observed ^{13}C-enrichment and ^{18}O-depletion in HL *O. universa* relative to specimens maintained in the dark are clearly a function of symbiont photosynthesis and have been reported before. With respect to ^{13}C-enrichment, a mechanism has been proposed whereby RUBISCO preferentially removes $^{12}CO_2$ during photosynthesis and subsequently enriches the calcifying environment with ^{13}C (Spero 1992; Spero and DeNiro 1987; Spero and Lea 1993; Spero et al. 1991; Spero and Williams 1989).

Because it is generally assumed that photosynthesis *per se* does not affect the $\delta^{18}O$ of the photosynthate nor that of the residual H_2O-CO_2-HCO_3^-pool (Swart 1983), ^{18}O-depletion in symbiotic systems were tentatively explained as the result of light-enhanced calcification rates (Bouvier-Soumagnac et al. 1986; Spero 1992; Spero and Lea 1993). However, as discussed before, our data suggest that calcification rate may not explain the oxygen isotope fractionation observed in this study. In the rest of this section we propose an alternative mechanism based on local pH changes induced by carbon fixation of the symbionts.

Since the symbiotic algae increase the pH of the foraminiferal microenvironment to as much as 8.8 during photosynthesis (Jørgensen et al. 1985), the local $[CO_3^{2-}]$ will be elevated as well (Wolf-Gladrow et al.1999). The observed differences in $\delta^{18}O$ between HL and dark range from 0.35 ‰ for *O. universa* (Spero 1992) to 0.63 ‰ for *G. sacculifer* (Spero and Lea 1993). If we assume that this difference is solely due to the symbiont induced shift in the ambient carbonate chemistry, and further that the $\delta^{18}O/[CO_3^{2-}]$ and a $\delta^{13}C/[CO_3^{2-}]$ slopes are -0.002 ‰ and -0.0065 ‰ respectively (Table 1), a simultaneous shift of 1.1 ‰ and 2.0 ‰ towards more negative $\delta^{13}C$ values at HL can be calculated for *O. universa* and *G. sacculifer*, respectively. On the contrary, we found a positive shift of 1.1 ‰ for *O. universa* and others have shown that compared to specimens grown in the dark, $\delta^{13}C$ of *O. universa* and *G. sacculifer* kept above saturation light levels, is enriched by 1.7 ‰ (Spero 1992) and 1.4 ‰ (Spero and Lea 1993), respectively. Consequently, if we take the kinetic effect on $\delta^{13}C$ and $\delta^{18}O$ (induced by local changes in the carbonate chemistry) into account, we have to invoke an additional mechanism to explain a concomitant enrichment in $\delta^{13}C$ between 2.2 to 2.8 ‰ for *O. universa* and even 3.4 ‰ for *G. sacculifer*.

RUBISCO has always been the first choice to explain shell enrichment in the light. However, this mechanism requires that sufficient CO_2 is available to enrich the residual CO_2 pool or, if HCO_3^- is the carbon source, that not all CO_2 (once converted from HCO_3^-) if fixed by RUBISCO and that some diffuses to the site of calcification. Numerical experiments (Wolf-Gladrow et al. 1999) have demonstrated that the symbionts are CO_2 limited, even at low photosynthetic rates, and therefore use HCO_3^- as their main carbon source. These experiments have also demonstrated that a steep CO_2 gradient exists between the shell surface and the symbiont halo, probably acting as an efficient barrier against CO_2 diffusion towards the shell. As the HCO_3^-pool is very large and hardly affected by photosynthetic carbon fixation, and the $\delta^{13}C$ of HCO_3^- is very close to the $\delta^{13}C$ of ΣCO_2, we argue that RUBISCO is probably not responsible for the HL:dark shift. This conclusion is supported by experiments carried out at constant pH (Fig. 3e). As CO_2 availability increases, shells get lighter instead of heavier, suggesting that enzyme mediated ^{13}C-enrichment of the ambient environment plays a subordinate role, if at all.

As an alternative hypothesis to explain the observed positive shift between the HL and dark experiments we propose scavenging of respired $^{12}CO_2$ by the symbionts. In the previous section we have shown that respired CO_2 can lower shell $\delta^{13}C$ by 3.2 to 3.8 ‰ (depending on the food source), enough to explain the required enrichment in the light of up to 3.4 ‰. The decrease in shell $\delta^{13}C$ with increasing $[CO_2aq]$ at constant pH can now be explained by a decrease in the scavenging efficiency of respired CO_2 as bulk CO_2 increases. Thus

scavenging of light respired CO_2 can explain the observed difference between the HL and dark results and the trend noticed in the constant pH experiments. In addition, effective scavenging of light respired CO_2 could also explain the very negative value of the symbiont organic matter (-24 ‰) even though HCO_3^- is the main carbon source.

The proposed mechanism of CO_2 scavenging can operate only if metabolic CO_2 diffuses freely through the membrane of the host and is not stored in an internal inorganic carbon pool. The pulse-chase experiments demonstrate that such a pool is not present in *O. universa* (Fig. 6).

Proposed Fractionation Mechanisms

Based on our experiments we propose that 3 fractionation mechanisms can be recognized:
1) pH or $[CO_3^{2-}]$ dependent kinetic fractionation of both carbon and oxygen stable isotopes.
2) Incorporation of respired CO_2 depleted in ^{13}C. In symbiont bearing species in the light.
3) Enrichment of the calcifying environment with ^{13}C (compared to the dark) due to scavenging of light respired CO_2 (RUBISCO related effects are probably of minor importance).

Light/dark Shift of Shell Stable Isotopes

In all experiments ($Calk_t$, $C\Sigma CO_2$ and CpH), the $\delta^{18}O$ value of shells grown in the light is depleted compared to shells kept in the dark. Such a decrease in shell $\delta^{18}O$ with increasing irradiance has been documented before for *G. sacculifer* and *O. universa* (Spero and Lea 1993). Because metabolic processes do not directly affect the $\delta^{18}O$ signal of the shell, we argue that an increase of pH or $[CO_3^{2-}]$ in the calcifying environment, either due to bulk carbonate chemistry changes or induced by photosynthesis, depletes shells in ^{18}O via mechanism 1.

The enrichment of $\delta^{13}C$ in the light observed in all experiments ($Calk_t$, $C\Sigma CO_2$ and CpH) must be explained in terms of competing processes. First is the depletion of shell $\delta^{13}C$ kinetically driven by the photosynthetically raised pH (mechanism 1). Secondly, Zeebe et al. (1999) have demonstrated that more respired CO_2 gets incorporated into the shell at higher pH. Hence, because the $\delta^{13}C$ of respired CO_2 is significantly depleted compared to bulk CO_2, the fractional change of metabolic versus bulk CO_2 incorporation into the shell will reinforce the depletion. In addition, a higher light- compared to dark-respiration rate of the symbionts (Weger et al. 1989) may deplete the calcifying environment even more under HL. The enrichment of shell $\delta^{13}C$ is dictated by very effective scavenging of light respired CO_2 (mechanism 3). A decline in the scavenging efficiency with increasing $[CO_2aq]$ could explain the trend in the constant pH experiments (more respired CO_2 escapes photosynthetic fixation and gets incorporated into the shell).

The question may be raised why scavenging of respired CO_2 dominates the impact of kinetic fractionation (mechanism 1) on $\delta^{13}C$ signal but not on the $\delta^{18}O$ signal? The reason is twofold. First, theoretical considerations require that the impact of kinetic fractionation on $^{13}C/^{12}C$ is half that of $^{18}O/^{16}O$ (McCrea 1950). Second, the relative depletion of respired CO_2 with respect to ^{13}C is large whereas the depletion in ^{18}O may be lost quickly due to the large water reservoir compared to ΣCO_2 and the fast exchange reactions.

Decoupling of the $\delta^{13}C/[CO_3^{2-}]$ Relationship at High $[CO_2aq]$ in the Light

A change in the scavenging efficiency may also explain the "plateau" or the decrease in $\delta^{13}C$ towards lower $[CO_3^{2-}]$ in the constant alkalinity experiments (Fig. 3a). At higher $[CO_2aq]$ (i.e. lower $[CO_3^{2-}]$), scavenging of metabolic CO_2 may become less effective and consequently an increasing portion of light respired CO_2 is incorporated into the shell. Again, the opposing affect of RUBISCO at high $[CO_2aq]$, i.e. the enrichment of the ambient ΣCO_2 with ^{13}C apparently plays a subordinate role. As noted before is the photosynthetic assimilation of carbon strongly CO_2 limited (Wolf-Gladrow et al. 1999). Apparently, even at the maximum PCO_2 reached in the constant alkalinity experiments (ca. 1900 µatm.) the symbionts are still CO_2 limited. In seperate experiments (Spero, Bijma and Lea unpubl results), PCO_2 was varied between 1400 µatm and more than 8000 µatm. The results

(not shown here) support the hypothesis that the decrease in shell $\delta^{13}C$ in the light should be explained in terms of a reduction in the scavenging efficiency and that RUBISCO is of secondary importance. The reason that the $\delta^{18}O$ signal under HL is not decoupled from $[CO_3^{2-}]$ at high PCO_2 (Fig. 3b) is because $\delta^{18}O$ of metabolic CO_2 and bulk CO_2 are probably not much different. Consequently, changes in the scavenging efficiency do not effect shell $\delta^{18}O$.

Conclusions

1) Shifts in pH (and for natural sea water in $[CO_3^{2-}]$) have a significant impact on foraminiferal shell $\delta^{13}C$ and $\delta^{18}O$.
2) Both symbiotic (*Orbulina universa*) and non-symbiotic (*Globigerina bulloides*) species display this effect. Therefore, we hypothesize that this effect is common among other species.
3) The effect is species dependent and based on a combination of kinetic and metabolic fractionation processes.
4) A pH/carbonate isotope effect has important implications for the interpretation of stable isotope data in the fossil record. Shells calcifying during cold climate periods record isotopic signatures that cannot be interpreted directly using present-day relationships (cf. Lea et al. this volume)
5) Because the carbonate ion effect appears to be a major factor influencing the foraminiferal isotope record, calibration of the response in each of the major paleoceanographic species is a necessary next step towards fully understanding and utilizing the oceanic isotopic record.

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