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Experimental determination of the ontogenetic stable isotope variability in two morphotypes of *Globigerinella siphonifera* (d'Orbigny)

Jelle Bijma [a,*], Christoph Hemleben [b], Brian T. Huber [c], Helmut Erlenkeuser [d], Dick Kroon [e]

[a] *Alfred-Wegener-Institut für Polar- und Meeresforschung, Columbusstraße, D-27568 Bremerhaven, Germany*
[b] *Institut und Museum für Geologie und Paläontologie, Universität Tübingen, Sigwartstr. 10, D-72076 Tübingen, Germany*
[c] *Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA*
[d] *Institut für Reine und Angewandte Kernphysik, C14-Labor, Universität Kiel, Max-Eyth-Str. 11, D-24118 Kiel, Germany*
[e] *Department of Geology and Geophysics, Grant Institute, University of Edinburgh, West Mains Road, EH9 3JW Edinburgh, UK*

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Abstract

Carbon and oxygen isotope fractionation in two morphotypes (Type I and Type II) of the symbiont bearing planktic foraminifer *Globigerinella siphonifera* (d'Orbigny) is investigated. SCUBA collected specimens were grown in the laboratory under identical culture conditions and their stable isotope signature was analyzed to characterize the influence of ontogeny and feeding rate on their $\delta^{13}C$ and $\delta^{18}O$ signals. The two types show a positive linear correlation between $\delta^{13}C$ and $\delta^{18}O$ with size. Type II is enriched in ^{13}C and ^{18}O relative to Type I and the enrichment per size increment is greater than for Type I. The carbon isotope composition of Type I tends towards lighter values at higher feeding rates whereas Type II is unaffected by the feeding regime. In order to determine if the isotopic response can be attributed to differences in growth characteristics and/or host/symbiont interactions, specimens were cultured under a variety of conditions and the pigment composition of freshly collected specimens was measured. Type I has a much lower photo-pigment content, which probably implies a lower gross photosynthetic rate. In addition, its growth and calcification rate are lower. The impact of these life processes on the stable isotope composition is discussed and it is argued that isotope fractionation is controlled by two linked processes. The carbon isotope fractionation is affected directly by a ^{12}C depletion or enrichment of the microenvironment via symbiont photosynthesis and host respiration, respectively. Concurrently, the life processes invoke a kinetic fractionation of the carbon and oxygen isotopes via their impact on the ambient carbonate chemistry.

Keywords: planktic foraminifera; biology; stable isotope fractionation; ontogeny

* Corresponding author. Present address: Fachbereich Geowissenschaften, Universität Bremen, Klagenfurter Strasse, D-28359 Bremen, Germany.

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1. Introduction

Carbon and oxygen isotopes of foraminiferal shells are routinely used in paleoceanography to reconstruct physical and biological properties of past oceans (e.g. temperatures, ice volumes, productivity). However, because the effects of host and symbiont physiology on the intra- and inter-specific isotope variability are not yet completely understood, it is difficult to discriminate vital effects from the environmentally induced variation. Hence, vital effects or metabolic fractionation, when not understood, greatly reduce the potential of isotope geochemistry as a paleoceanographic tool. In this study, we use an experimental approach to decipher the processes that control the isotopic fractionation and Type-specific variability in *Globigerinella siphonifera*.

Globigerinella siphonifera is a symbiont bearing, spinose planktonic foraminifer that inhabits the mixed layer of oligotrophic, tropical to subtropical water masses. Two types, designated Type I and Type II, are distinguished based on their genetic, morphological, physiological and chemical differences (Table 1). In the field, the two types can readily be identified by SCUBA divers (Faber et al., 1988, 1989). Type I is rather conspicuous having numerous whitish spines and a light brown shell. If *Trichodesmium* sp. is present in the ambient water, almost all Type I will be found in association with these blue-green bacteria. Type II is more difficult to discern in the water because the shell and the spines are much darker and the spine density is lower. Type II has never been observed to associate with *Trichodesmium*. Under the inverted light microscope, the rhizopodial network of Type I is strongly reticulate and dispersed between the spines, whereas the rhizopodia of Type II are only weakly reticulate and mainly run parallel to the spines. Because the symbionts are associated with the cytoplasmic strands and the rhizopodial network of Type I is web-like, the symbionts are dispersed between the spines. In contrast, in Type II the symbionts are lined up along the spines and are found in the vicinity of or inside the shell, explaining the darker colour of the spines and the shell (for light micrographs see Huber et al., 1997, fig. 2). In addition, Type I is frequently found

Table 1
Major observational and geochemical differences between *G. siphonifera* Type I and Type II

	Type I	Type II	Reference
Generic			
Test color	pale	dark	Faber et al., 1988; this study
Rhizopodial network	web-like	along spines	Faber et al., 1988; this study
Trichodesmium sp.	common	very rare	Faber et al., 1988; this study
Dinoflagellate 'swarmers'	common	generally absent	Faber et al., 1988; Huber et al., 1997
Spine density	higher	lower	this study
Empty shell			
Test shape	relatively evolute	relatively involute	Huber et al., 1997; this study
Average mature test size	549 μm	449 μm	Huber et al., 1997; this study
Kummerform frequency	4%	18%	this study
Average adult porosity	10–30%	4–10%	Huber et al., 1997
Average adult pore diameter	4.5 μm	2.2 μm	Huber et al., 1997
Biology			
Symbiosis	facultative	obligatory	this study
Symbiont number	lower	higher	Faber et al., 1988
Total pigment content	3.2 ng/host	17.0 ng/host	this study
Average longevity in culture	11.5 days	8.3 days	this study
Chamber-form. rate	0.3/day	0.4/day	this study
Shell chemistry			
$\delta^{13}C$ offset from equil. (size fraction >300 μm)	−2.85‰	−2.08‰	this study
$\delta^{18}O$ offset from equil. (size fraction >300 μm)	−0.66‰	−0.31‰	this study
Mg/Ca offset vs. Nürnberg et al., 1996b	0.61 (mM/M)	0.05 (mM/M)	this study

with heterotrophic dinoflagellate swarmers between the spines, whereas we have observed them in only a few Type II specimens.

The symbionts sequestered within each type of host show prominent differences (Faber et al., 1988) and were reported to significantly affect growth and longevity of the host in culture (Faber et al., 1989). Type II holds more symbionts than Type I at comparable size and the symbionts associated with Type II contain more chloroplasts per alga than Type I (Faber et al., 1988). Faber et al. (1988) concluded, on the bases of chloroplast location, position of the large vacuole and spacing of the thylakoid, that the symbionts of Type I and Type II are probably different species of chrysophytes. Gastrich (1988), however, concluded that the symbionts belong to the same species and are more likely to be haptophytes because the chloroplasts lack a girdle lamella.

Although living specimens can easily be identified, the empty shells of the two types are difficult to distinguish. Biometric study has revealed statistically significant, but visually subtle, differences in pore size and chamber arrangement in the *G. siphonifera* morphotypes, with Type I shells attaining much greater shell porosity and more evolute coiling than Type II (Huber et al., 1997). Since the compared specimens were collected at the same time and at the same water depth, it is unlikely that types I and II are ecophenotypes. This assertion is supported by DNA sequencing of types I and II, which revealed that these morphotypes should be considered as sister species (Darling et al., 1997). Huber et al. (1997) concluded that types I and II are examples of cryptic speciation, whereby biological speciation has occurred in the absence of discernable change in shell morphology.

2. Materials and methods

2.1. Collection and culture procedure

All experiments were carried out at the Caribbean Marine Biological Institute (CARMABI), Curaçao, Netherlands Antilles. Specimens were hand collected by SCUBA divers from a depth of 3–6 m, two miles off the west coast of Curaçao (12ºN, 69ºW). After collection, the foraminifers were brought back to the CARMABI, where the general condition of each specimen was inspected and the maximum shell diameter was measured. Specimens between 150 and 340 μm wide were selected for culture and care was taken that each experimental group had a similar average shell diameter. In all experiments foraminifers were grown individually in glass culture vials with approximately 40 ml medium and a plastic cap (to prevent evaporation). We used flat-bottomed glass vials (Ace Scientific 10-5032-30). These were acid washed, rinsed three times with distilled water and dried in an oven at 80ºC. Before use, they were rinsed with the culture medium.

The culture medium was 0.45 μm filtered sea water (FSW) from the collection site. Specimens were transferred with a wide mouth pipette into the culture vials and placed in a constant temperature bath. Cultures were illuminated from above (diel cycle: 12 h light/12 h dark) with one of two different light regimes: (1) A low light intensity regime of ca. 60 $\mu E\ s^{-1}\ m^{-2}$ (L1) with a mixture of blue and white light bulbs (Osram 40W-64, Philips TL 40W-55). This regime simulated illumination in the ocean at a depth between 20 and 30 m and mainly emitted light of 430–470 nm wavelength (Hemleben and Spindler, 1983; Spindler et al., 1984). (2) A high light intensity regime of ca. 160 $\mu E\ s^{-1}\ m^{-2}$ (L2) with white light bulbs only (Philips TL 40W-55). This regime corresponds to a depth of approximately 15 m at the study site but the spectral quality of the bulbs does not simulate the spectral quality of the light at 15 m.

The specimens were fed freshly hatched brine shrimp (BS = *Artemia* nauplii, San Francisco Bay strain). All specimens were observed daily, using an Olympus inverted microscope, to record shell size, the condition of the spines and rhizopodia. Maximum shell diameter was measured to detect new chamber formation and the shape of new chambers (kummerform or normal) was recorded.

Under normal growth conditions *G. siphonifera* adds 3 or 4 chambers in culture before gametogenesis. During this process, the spines are shed and the cytoplasm is converted into gametes, leaving an empty shell. Healthy specimens usually undergo gametogenesis 6–8 days after collection. By contrast, unhealthy specimens can stay alive much longer and die without undergoing gametogenesis. Their shells

remain spinose and filled with cytoplasm. Thus, in order to distinguish between gametogenesis and death without reproduction, the absence or presence of spines was monitored. The survival time of non gametogenetic specimens was determined by the halting of cytoplasmic streaming in the rhizopodia.

In 1988 we started experiments to investigate growth characteristics and longevity of types I and II under different culture treatments. At a later stage, in 1990, we carried out experiments to investigate differences with respect to the isotopic fractionation of the two types. For reference purposes, we analysed the isotopic composition of freshly collected types I and II (1992 and 1994). Specimens collected in 1994 were also used to analyze the pigment composition of the symbionts and to determine Mg/Ca ratios of the shell.

2.2. Growth experiments

During February through June 1988 the mean temperature and salinity values at the collection site were 27.2±0.5ºC and 36.6±0.3‰, respectively. The culture water was maintained at 23.5 ± 0.5ºC. Experiments were designed to separate host and symbiont effects on growth characteristics. To investigate the impact of the foraminifer on growth and survival, experiments were carried out under low light intensity (L1) and at a feeding rate of 1 brine shrimp per day (1 BS/d). To investigate the impact of the symbionts, specimens were grown at a lower feeding schedule (1 BS/2d), increased light intensity (L2) and in simulated eutrophic nutrient concentrations. Enriched filtered sea water (EFSW) was prepared by adding 0.93 ml enrichment stock solution to 1 l FSW. The stock solution contained 4.667 g/l $NaNO_3$, 0.667 g/l Na_2-glycerophosphate and 0.065 g/l NH_4Cl. Thus EFSW was enriched with ~51 μmol/l NO_3^-, ~2.0 μmol/l PO_4^{2-} and ~1.1 μmol/l NH_4^-. Finally, four different combinations of culture conditions were chosen to investigate growth characteristics of Type I and Type II to abiotic (nutrient level and light regime) and biotic (feeding rate) variables:

(1) FSW, L1, 1 BS/d;
(2) EFSW, L1, 1 BS/d;
(3) FSW, L2, 1 BS/2d;
(4) EFSW, L2, 1 BS/2d.

For statistical analysis, only specimens that underwent gametogenesis between 4 to 15 days after collection were used. Additional criteria were an initial size <340 μm and at least two chamber additions in the laboratory. From a total of 433 cultured specimens, only 263 met these criteria. The results are listed in Table 2. The chamber formation rate was calculated by dividing the number of chambers formed by the longevity. The calcification rate was calculated by subtracting the initial from the final shell weight (using the size–weight relationship, Fig. 1) and dividing it by the longevity.

2.3. Stable isotope experiments

To minimize the effect of bacteria and algae on $\delta^{13}C$ of dissolved inorganic carbon (DIC) in the cultures, the foraminifers were grown in autoclaved FSW. For this, 10 l of sea water from the collection site was filtered over a 0.45 μm nucleopore filter and autoclaved in large glass containers with a cotton plug. Upon cooling, flaky iridescent crystals formed but salinity remained largely unaffected (EDAX analysis showed that small amounts of Ca and Mg salts had precipitated). The medium was filtered again over a 4 μm Whatman filter, stored in a 25 l acid washed black, plastic container and aerated overnight to equilibrate with the atmosphere. The $\delta^{13}C$ of the culture medium was monitored on a daily basis (100 ml medium was collected in an airtight serum bottle and poisoned with 1 ml saturated $HgCl_2$ solution). To maintain a constant $\delta^{13}C_{DIC}$ during culture, specimens were transferred to clean culture vessels containing fresh medium in the early morning and in the late afternoon using a wide-mouth pipette. Foraminifers were maintained under L1, at 26.5ºC ± 0.5ºC and fed at a rate of 1 BS/2d, 1 BS/d or 2 BS/d. After the addition of 2 chambers, they were killed with a quick rinse in distilled water, dried and archived in micropaleo-slides for later isotope analysis. We did not amputate the chambers grown in culture but analysed whole specimens. The number of specimens used for stable isotope analysis depended on shell length and varied between 3 and 17.

Reference samples were collected by SCUBA, killed in distilled water and dried immediately for later isotope analysis. Types I and II specimens collected in 1992 were analysed as whole specimens.

Table 2
Growth characteristics of *G. siphonifera* Type I and Type II to different laboratory treatments

Group	Type	Specimens (#)	Medium	Light	BS feeding rate (BS/d)	BS acceptance rate (BS/d)	Mean initial size ± sd (μm)	Mean final size ± sd (μm)	Growth ± sd (μm)	Longevity (d)	Chambers formed (#)	Chamber formation rate (#/d)	Calcification rate (nmol/d)
1	I	45	FSW	L1	1	0.90 ± 0.13	267 ± 41	621 ± 59	354 ± 72	10.2 ± 2.1	3.7 ± 0.8	0.36	8.8 ± 4.0
2	II	15	FSW	L1	1	0.80 ± 0.28	271 ± 77	560 ± 94	289 ± 112	7.8 ± 2.4	3.7 ± 1.3	0.47	11.9 ± 5.7
3	I	65	EFSW	L1	1	0.88 ± 0.15	279 ± 51	630 ± 78	351 ± 86	11.7 ± 2.4	3.5 ± 0.8	0.30	8.8 ± 5.1
4	II	9	EFSW	L1	1	0.60 ± 0.18	258 ± 76	559 ± 100	301 ± 82	9.2 ± 3.2	3.7 ± 1.2	0.40	12.3 ± 8.4
5	I	34	FSW	L2	0.5	0.45 ± 0.08	252 ± 42	530 ± 64	278 ± 64	12.1 ± 2.2	3.1 ± 0.8	0.26	3.7 ± 1.5
6	II	27	FSW	L2	0.5	0.46 ± 0.11	269 ± 48	514 ± 74	246 ± 68	7.9 ± 2.1	3.0 ± 0.7	0.38	7.9 ± 4.0
7	I	38	EFSW	L2	0.5	0.47 ± 0.05	271 ± 42	540 ± 46	269 ± 51	11.9 ± 2.2	3.0 ± 0.8	0.25	3.9 ± 1.3
8	II	30	EFSW	L2	0.5	0.46 ± 0.08	277 ± 39	525 ± 54	248 ± 58	8.1 ± 1.9	2.9 ± 0.7	0.36	7.9 ± 3.2

Specimens were cultured in filtered seawater (FSW) and in nutrient enriched filtered seawater (EFSW), under blue/white light of 60 μE s^{-1} m^{-2} (L1) or under white light of 160 μE s^{-1} m^{-2} (L2). The values for shell size, growth, survival time, number of chambers formed, chamber formation rate and calcification rate are means ± the standard deviation.

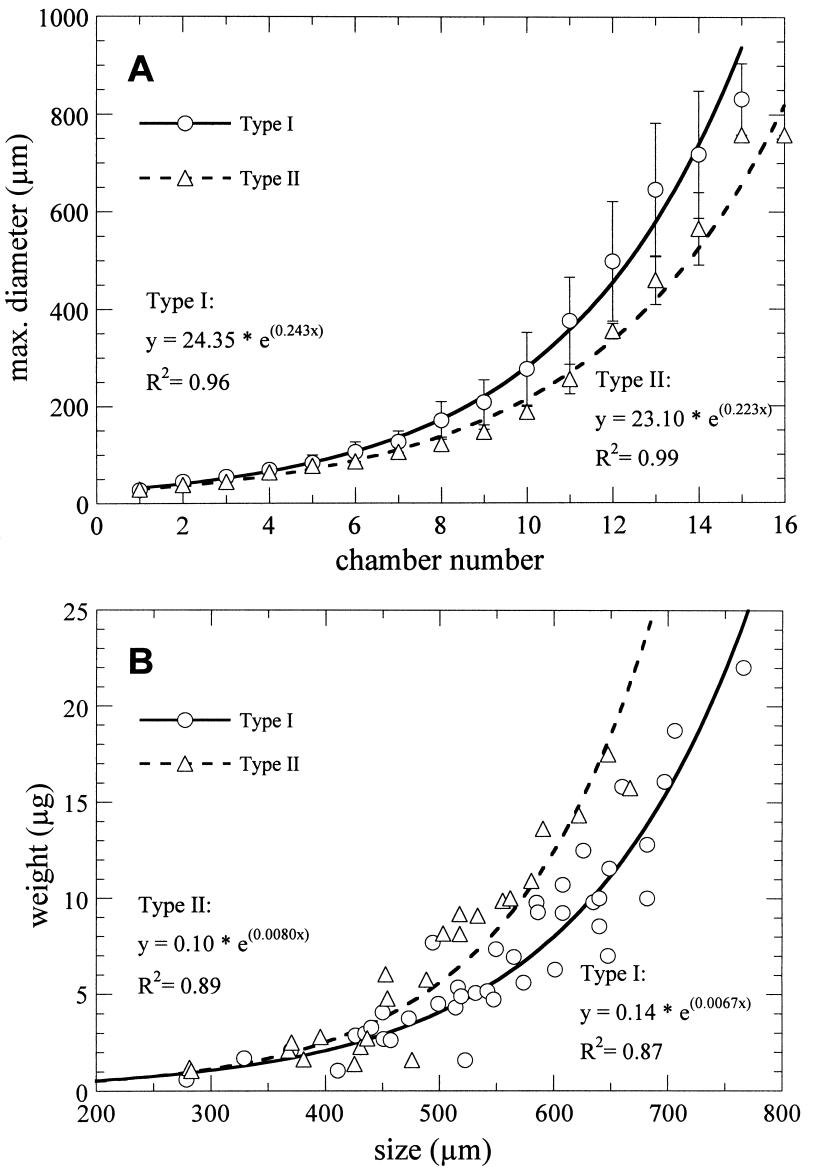

Fig. 1. (A) The chamber–size relationship of *G. siphonifera* Type I and Type II determined for translucent specimens. (B) The size–weight relationship of *G. siphonifera* Type I and Type II. Samples were weighted on a micro-balance or the weight was determined on the basis of the CO_2 yield in the mass spectrometer. The data were fitted by ordinary least squares.

The temperature at the collection site was 26.4ºC. The chambers of specimens collected in 1994 (at 26.8ºC) were amputated until the remaining shells were approximately 300 µm. The amputated chambers were pooled and the remaining shells were pooled with whole shells <300 µm for isotope analysis.

2.4. Isotope analysis

All foraminiferal samples were roasted in a low temperature asher (LTA 302) prior to isotopic analyses. The shells were analysed in East Kilbride (Edinburgh) on a VG Prism (samples from the 1990 experiments) and in Bremerhaven on a Finnigan mass spec-

trometer with a 'Kiel device' (samples from 1994). The precision for $\delta^{18}O$ is about 0.08‰ and for $\delta^{13}C$ is about 0.06‰ for both machines. All isotopic measurements are reported relative to the Pee Dee Belemnite (PDB) standard (unless stated differently) using conventional δ notation (Craig, 1957).

Water samples were measured on a Finnigan mass spectrometer in Kiel (Erlenkeuser and Party, 1995). The precision for $\delta^{13}C_{DIC}$ is about 0.1‰. The $\delta^{13}C$ of the DIC at the collection site was 1.25 ± 0.12‰ ($n = 20$). After autoclaving the $\delta^{13}C_{DIC}$ of the water was 2.46 ± 0.53‰ ($n = 24$) and thus enriched by 1.2‰. Equilibration overnight depleted the $\delta^{13}C_{DIC}$ of the medium by 0.3‰ ($n = 9$). Consequently, the culture water was enriched by 0.9‰ with respect to water at the collection site. Unfortunately, the $\delta^{13}C_{DIC}$ of the stock solution, and thus that of the culture medium, was depleted by a few tenths of a permil over the course of the experiments. This shift is probably due to the build up of respired CO_2 (depleted in ^{13}C) in the laboratory and the presence of a head space in the culture vials (Spero and Lea, 1993). Since the $\delta^{13}C_{DIC}$ of the culture medium was not constant we can only speculate about the $\delta^{13}C$ composition of equilibrium calcite. However, because the experimental treatments were carried out in parallel and the number of shells combined for each isotopic analysis was relatively high, we can compare between Type I and Type II and between the different feeding regimes.

The oxygen isotopic composition of the culture water was not monitored, but experiments carried out at the AWI have demonstrated that autoclaving raises the $\delta^{18}O$ by 0.15 ± 0.05‰ on the SMOW scale ($n = 9$).

2.5. Mg/Ca analysis

In 1994 we collected specimens to determine the Mg/Ca ratios of the shells. The temperature at the collection site was 26.8ºC. Using an electron microprobe, the Mg/Ca ratio was determined for freshly collected types I and II according to the method of Nürnberg (1995). To calculate the apparent calcification temperature for types I and II we used the empirical relationship between culture temperature and the Mg/Ca ratio in the shells of planktic foraminifera developed by Nürnberg et al. (1996a,b).

2.6. Pigment analysis

To analyze the pigment composition of the symbionts, specimens were collected by SCUBA. Quantitative and qualitative pigment measurements were carried out on methanol extracts of crushed but not dried foraminifers. Ninety-nine specimens of Type I and 43 specimens of Type II were collected by SCUBA and measured. The procedure for the pigment analysis generally followed that of Strickland and Parsons (1972) with modifications reported by Kuile and Erez (1984). Once measured, the foraminifers were transferred to centrifuge tubes and stored in the dark at −20ºC.

For analysis:

(1) the foraminifers were crushed with a micro mortar;

(2) the pigments were extracted with 5 ml 100% methanol for 4 h (in the dark), mixing with a vortex stirrer at the start and after 2 h;

(3) during transfer to the centrifuge, the tubes containing the mixture were wrapped in tin foil;

(4) the mixture was centrifuged for 10 minutes at 7000 r.p.m.;

(5) the absorbency of the supernatant was measured at 750, 665, 647, 630, 510, and 480 nm on a Beckman DU-65 spectrophotometer.

The pigment concentration (in mg l^{-1}) was calculated using the following equations from Parsons et al. (1984):

Chlorophyll *a*

$$= 11.85 \cdot E_{664} - 1.54 \cdot E_{647} - 0.08 \cdot E_{630} \quad (1)$$

Chlorophyll *c*

$$= 24.52 \cdot E_{630} - 1.67 \cdot E_{664} - 7.60 \cdot E_{647} \quad (2)$$

$$\text{Carotenoid} = 7.6 \cdot (E_{480} - 1.49 \cdot E_{510}) \quad (3)$$

where E stands for the absorbency at different wavelengths (corrected by the 750 nm reading). For qualitative pigment analysis, the absorbency in the range 400–700 nm was determined in 10 nm steps, starting at the low energy side (700 nm) of the spectrum.

2.7. Size–weight relationships

Foraminifers grow by addition of separate chambers (accretionary growth). Using lightly calcified

(translucent) specimens, the chamber-by-chamber increase in maximum shell diameter was measured under the inverted microscope. Weights were determined on a Mettler microbalance or on the basis of the CO_2 yield (in volts) of the mass spectrometer. The precision of the two methods is comparable and about 1 μg.

3. Results

3.1. Field data

Type I is larger than Type II at comparable ontogenetic stages (Fig. 1A). At equal size, however, Type II is heavier than Type I (Fig. 1B). We recorded fewer kummerform chambers for Type I (4%) than for Type II (18%).

3.1.1. Pigment data

The pigment scan between 400 and 700 nm (Fig. 2) shows that the two types possess similar pigments but that the chlorophyll *a* and *c* concentration in Type I is much lower (Table 3). Although the mean size of Type I was almost 200 μm larger than Type II, the first contained almost five times less chlorophyll *a* and *c*. Because the number of symbionts is positively related to the size of the host (Spero and Parker, 1985), we expect that, at equal size, Type I will have significantly less than five times lower chlorophyll *a* and *c* content as Type II. The chlorophyll *a*/carotenoid ratio in Type I, however, was 28% higher than in Type II (Table 3).

Table 3
Photopigment concentrations for Type I (99 specimens; mean size = 521 μm) and Type II (43 specimens; mean size = 338 μm)

Pigment	Type I		Type II	
	(μg/ml)	(ng/foram)	(μg/ml)	(ng/foram)
Chl *a*	0.08	1.61	0.16	7.41
Chl *c*	0.02	0.45	0.04	2.06
carotenoid	0.06	1.17	0.16	7.50
Chl *a*/car	1.38		0.99	

3.1.2. Stable isotopes

During February 2–May 7 1990, the temperature at the collection site increased from 25.7 to 27.1ºC (mean 26.3 ± 0.5ºC) while salinity decreased from 36.8 to 36.0‰ (mean 36.3 ± 0.3‰). The mean $\delta^{13}C$ of the total dissolved inorganic carbon (DIC) of the water was 1.25 ± 0.12‰. Because the $\delta^{13}C$ of calcite precipitated in isotopic equilibrium is 1‰ heavier than $\delta^{13}C_{DIC}$ (Romanek et al., 1992), the $\delta^{13}C$-equilibrium value for freshly collected specimens is 2.25‰ (Fig. 3A). For the western equatorial Atlantic a $\delta^{18}O$ value of 0.93‰ on the SMOW scale

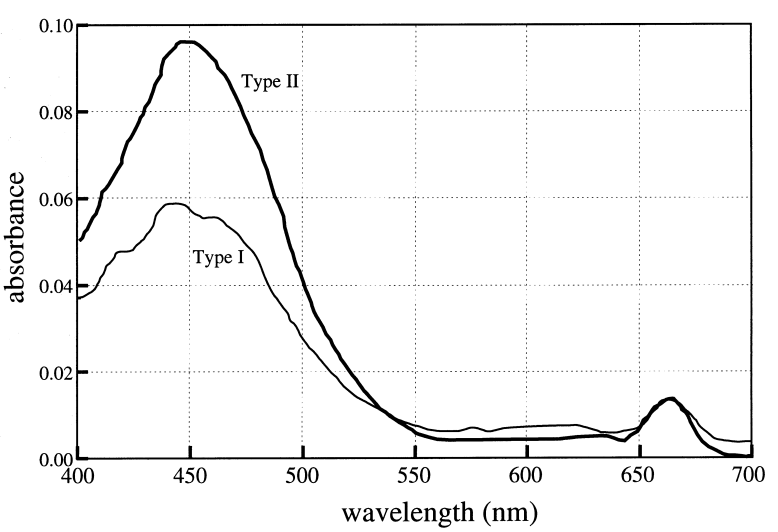

Fig. 2. Absorbency as a function of wavelength for Type I and Type II. For reasons of comparison, the absorbency at 664 nm were matched.

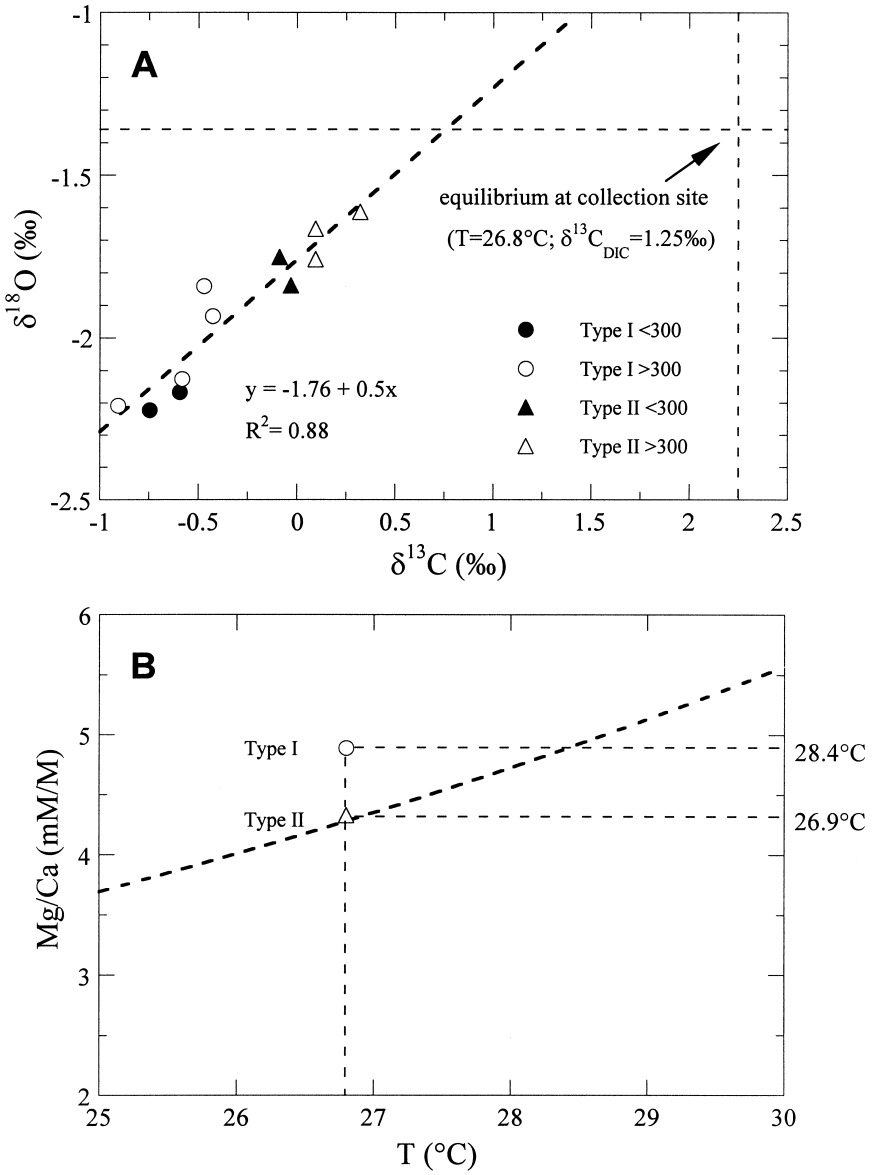

Fig. 3. (A) $\delta^{13}C$ vs. $\delta^{18}O$ for SCUBA collected shells <300 μm and pooled chambers of shells >300 μm of Type I and Type II. (B) The average Mg/Ca ratio in mM/M for all chambers for Type I and Type II. The fat broken line is the empirical relationship between temperature and Mg/Ca of Nürnberg et al. (1996b). The temperature at the collection site was 26.8ºC and the calculated 'equilibrium temperatures' of Type I and II are given on the secondary *Y*-axis.

can be calculated for $S = 36.3‰$ (Fairbanks et al., 1992). This is in excellent agreement with the value of 0.92‰ (SMOW) given for Caribbean water (Shackleton et al., 1973). At 26.4 and 26.8ºC (mean collection temperatures in 1992 and 1994, respectively) this is equivalent to a $\delta^{18}O$ value of −1.29 (Fig. 4B) and −1.37‰ (Fig. 3A), respectively, for equilibrium calcite (O'Neil et al., 1969). Apparently, *G. siphonifera* precipitates calcite out of equilibrium but values closer to equilibrium calcite are attained in later ontogeny (Fig. 3A and Fig. 4A, B).

The life cycle of most planktic foraminifera is

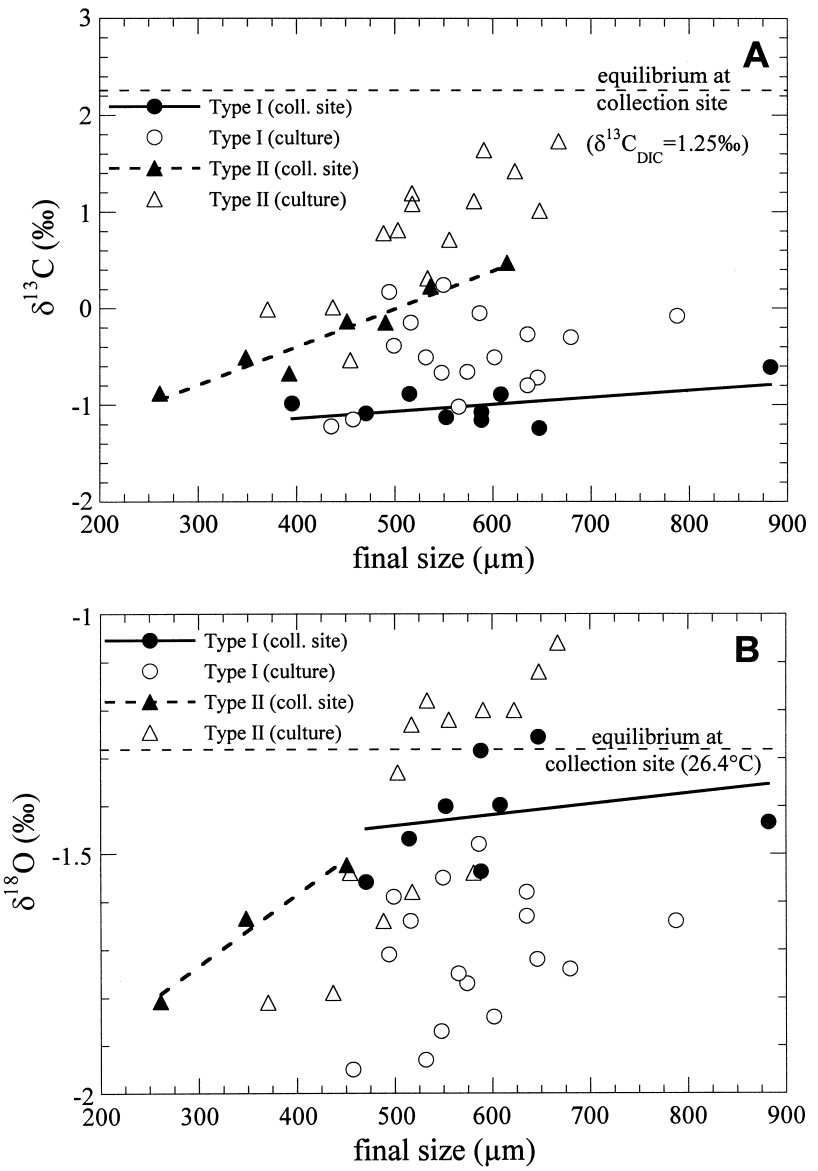

Fig. 4. The carbon (A) and oxygen (B) isotope ratio for Type I and Type II. The fat broken and solid lines are the regressions for the reference samples from the collection site (closed symbols). Open symbols are the data for specimens after secreting two chambers in culture. Calcite secreted in isotope equilibrium with respect to the $\delta^{13}C_{DIC}$ at the collection site is 2.25‰; calcite secreted in oxygen isotope equilibrium with sea water at the collection site is −1.29‰.

tuned to the lunar cycle during which they undergo ontogenetic migration (Bijma et al., 1990). The $\delta^{18}O$ values for both types suggest a migration from a shallow towards a deeper habitat in late ontogeny. Type II is enriched with ^{18}O and ^{13}C in comparison to Type I and displays a greater increase towards heavier values with ontogeny (Fig. 3A and closed symbols in Fig. 4). The heavier values and the greater $\delta^{18}O$-range for Type II suggests a deeper habitat and a larger ontogenetic depth range. Using the paleotemperature equation of O'Neil et al. (1969), the $\delta^{18}O$ value of Type II >300 µm (−1.68 ± 0.07‰)

can be converted to an apparent calcification temperature of 28.4 ± 0.4ºC whereas the larger fraction of Type I (-2.03 ± 0.17‰) suggests a calcification temperature of 30.2 ± 0.9ºC. Hence, the average difference in calcification temperature between Type I and Type II is 1.8ºC.

3.1.3. Mg/Ca ratios

The Mg/Ca molar ratios that were determined for types I and II are 4.89×10^{-3} and 4.33×10^{-3}, respectively (Fig. 3B). Using the relationship of Nürnberg et al. (1996b) and assuming that Type II secreted it's shell in equilibrium, the difference in the Mg/Ca ratio between types I and II would indicate a 1.5ºC warmer precipitation temperature for Type I than for Type II. These results are in close agreement with the observed $\delta^{18}O$ differences.

3.2. Laboratory cultures

In an unsuccessful attempt to rid specimens of their symbionts by culture in continuous darkness (for cross-infection experiments) we made an interesting observation. In the dark, Type I loses most of it's symbionts but after a few days in a normal light/dark cycle the number of symbionts steadily grows back to normal. By contrast, Type II did not survive more than a few days of continuous darkness, suggesting that the symbionts play a more important role for survival of Type II (obligatory symbiosis).

3.2.1. Growth experiments

Analyses of variance (multivariate statistics) was performed between comparable groups to test differences in growth characteristics (Table 4). The independent variables were Type (I or II), light and feeding conditions (L1 and 1 BS/d or L2 and 0.5 BS/d), and culture medium (FSW or EFSW). The mean initial sizes ranged between 252 and 279 μm for Type I and between 258 and 277 μm for Type II and were not significantly different for all treatments. Under the high feeding regime (1 BS/d), the brine shrimp acceptance rate (mean number of accepted BS divided by the longevity) was always higher for Type I than for Type II in comparable experiments (Table 2), suggesting that Type II can not fully exploit high feeding levels and that Type I is better adapted to handle living prey, probably because of the higher spine density. In all treatments, Type I is more successful at reaching gametogenesis than Type II (Table 2). In addition, more chambers were formed and larger final sizes were attained in Type I under high rather than under low feeding frequencies. Although the differences in the chamber formation rate between types I and II was significant in all treatments, the difference in final size was only

Table 4
Results of the analyses of variance between comparable groups of *G. siphonifera*, expressed as the computed *F*-ratios

Groups	Independent variables			Dependent variables					
	Type	Light/FR	Medium	Initial size	Final size	Growth	Longevity	Chamber formation rate	Calcification rate
1,2	*V*	L1/1	FSW	0.07 NS	8.26 **	6.52 ***	13.69 *	10.52 **	5.27 ***
3,4	*V*	L1/1	EFSW	1.15 NS	5.81 ***	2.56 NS	7.59 **	10.11 **	3.10 NS
5,6	*V*	L2/0.5	FSW	1.95 NS	0.79 NS	3.55 NS	55.22 *	21.23 *	31.14 *
7,8	*V*	L2/0.5	EFSW	0.32 NS	1.42 NS	2.29 NS	54.36 *	28.80 *	46.82 *
1,5	I	*V*	FSW	2.47 NS	41.18 *	22.98 *	14.20 *	25.59 *	47.74 *
2,6	II	*V*	FSW	0.02 NS	2.90 NS	2.29 NS	0.03 NS	4.05 NS	6.83 ***
3,7	I	*V*	EFSW	0.66 NS	41.10 *	27.93 *	0.16 NS	6.88 **	33.12 *
4,8	II	*V*	EFSW	0.92 NS	1.68 NS	4.45 ***	1.61 NS	2.32 NS	5.22 ***
1,3	I	1	*V*	1.76 NS	0.42 NS	0.04 NS	10.59 **	10.70 *	0.00 NS
2,4	II	1	*V*	0.15 NS	0.00 NS	0.07 NS	1.39 NS	0.66 NS	0.01 NS
5,7	I	0.5	*V*	3.63 NS	0.51 NS	0.49 NS	0.18 NS	0.13 NS	0.29 NS
6,8	II	0.5	*V*	0.50 NS	0.38 NS	0.02 NS	0.11 NS	0.62 NS	0.00 NS

Light and feeding regimes as described in Table 2. V = the independent variable, * = $p \leq 0.001$, ** = $p \leq 0.01$, *** = $p \leq 0.05$, NS = not significant; FR = feeding rate (B5/d).

significant under the low light, high feeding regime (Table 4). Apparently, under high light intensities Type II can better handle a lower feeding regime than Type I (i.e. their symbionts partly compensate the reduction in final shell).

The most striking difference was the discrepancy in life span between the two types. Type I had a significantly and consistently, longer survival time in all treatments, particularly under high light and a low feeding regime (on the average 1.4 times longer than that of Type II). Related to this, the rates of chamber formation and calcification of Type II were significantly higher than those of Type I in all but one experiment (although the difference in calcification rate between groups 3 and 4 was almost significant: $p = 0.08$). On average, the mean chamber formation rate was 1.3× and 1.5× higher under high and low feeding rates, respectively. The higher calcification rate for Type II is the result not only because of its shorter survival time but also because of its heavier shell (Fig. 1B). On the average, the mean calcification rate of Type II was 1.4× and 2.1× higher under high and low feeding conditions, respectively. Apparently, the difference in chamber formation rate and calcification rate between types I and II are more pronounced under a low feeding rate and high light intensities. Significant differences were also found with light/feeding regime as the independent variable. The calcification rate was significantly higher under low light and high feeding rates for both Types. The chamber formation rate was only significantly higher under those conditions for Type I. The addition of nutrients did not produce significant differences in the growth characteristics of each type.

3.2.2. Isotope experiments

The experiments demonstrate that for both carbon and oxygen isotopes, Type II is heavier and the ontogenetic enrichment is faster than for Type I. In culture, the $\delta^{13}C$ value of Type I ranges between −1.2 and 0.3‰ whereas for Type II the range is between −0.6 and 1.8‰ (Fig. 4A). The $\delta^{18}O$ value of Type I ranges between −2.0 and −1.5‰ whereas for Type II the range is from −1.8 to −1.1‰ (Fig. 4B). The reference samples (Fig. 3) confirm that Type II is heavier than Type I for both isotope species but do not show a steeper ontogenetic trend for Type II.

The experimental $\delta^{13}C$ values are enriched compared to the reference (on the average 0.5 and 1‰ for types I and II, respectively). Type I does not reflect the full 0.9‰ difference in $\delta^{13}C_{DIC}$ between collection site and culture water. This can be explained because we analysed whole specimens and part of the calcite was thus formed in the field. In addition, the $\delta^{13}C_{DIC}$ of the culture water depleted slightly over the course of the experiments. The reason why we do not observe this in Type II is probably because under controlled laboratory conditions it adds more calcite in a shorter time.

The small enrichment in $\delta^{18}O$ caused by autoclaving is lost in the scatter of the data. The average $\delta^{18}O$ value of Type II after culture is 0.27‰ heavier than the average reference value. We have no explanation why the average $\delta^{18}O$ of cultured Type I is depleted by 0.29‰ as compared to the reference data. At 26.5ºC (culture temperature) and assuming 0.15‰ enrichment due to autoclaving, equilibrium calcite should be −1.16‰ (O'Neil et al., 1969).

The experiments further demonstrate that the $\delta^{13}C$ value of Type I decreases as feeding rate increases (Fig. 5A) whereas the carbon fractionation of Type II did not change systematically with feeding level (Fig. 5B). Growth in continuous darkness decreases the $\delta^{13}C$ value of Type I shells by an average of 0.5‰. Because Type II did not form chambers in continuous darkness but underwent gametogenesis (90%) or died (10%) after 3–4 days, we were unable to obtain data under those conditions.

The oxygen isotope composition seems independent of the feeding regime, except for the low feeding levels. Type I becomes slightly enriched whereas Type II becomes slightly depleted when kept at a feeding scheme of 1 BS every two days (Fig. 6A, B). The $\delta^{18}O$ values for Type I maintained in the light or in continuous darkness were similar.

4. Discussion

Types I and II were shown to carry very different stable isotope signatures in the field and display a significantly different isotope fractionation behavior in the laboratory. Thus difficulties associated with distinguishing between fossil shells of the two types will limit our ability to interpret the geochemical

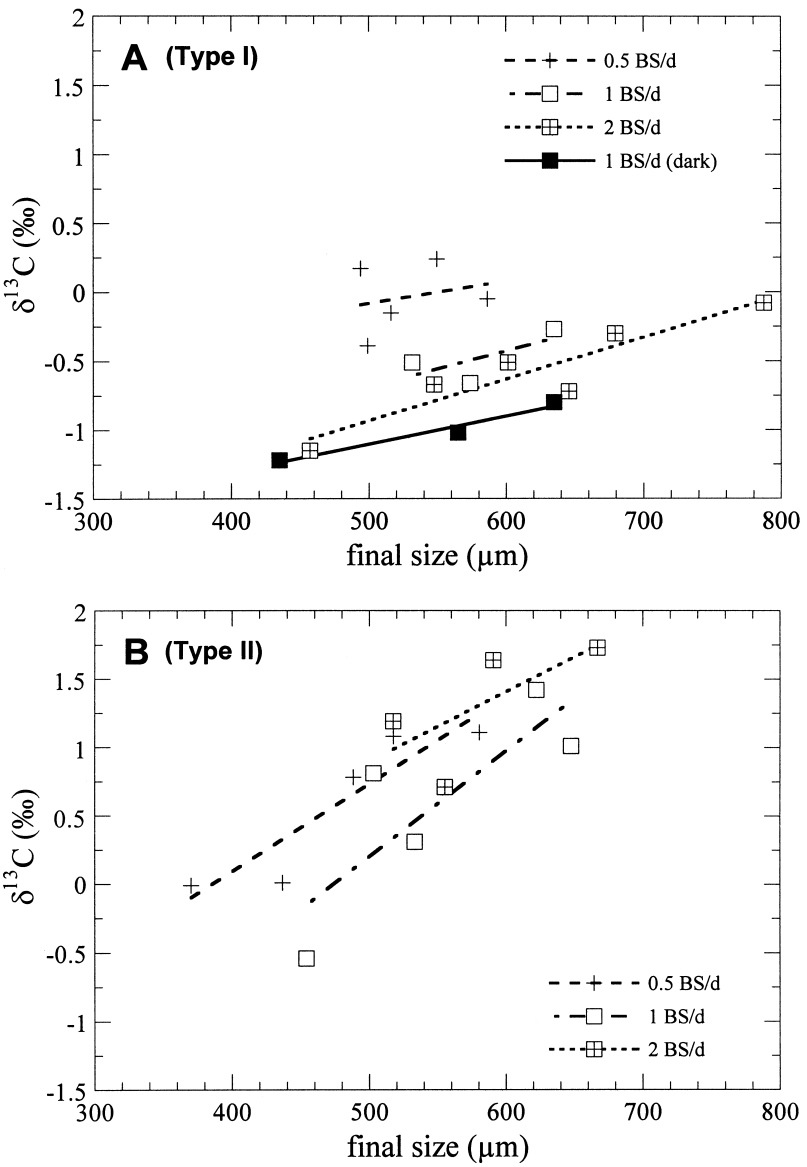

Fig. 5. The carbon isotope ratio of *G. siphonifera* Type 1 (A) and Type II (B) that formed two chambers in culture at three different feeding schedules. All experiments were carried out under L1. Type I also formed chambers in continuous darkness (closed square) whereas Type II underwent gametogenesis or died in continuous darkness without forming chambers.

information contained in the *G. siphonifera* isotopic record. On the other hand, the isotopic differences between the two morphotypes can contribute to our understanding of the mechanisms underlying disequilibrium fractionation. Several laboratory studies have been dedicated to a better understanding of isotope fractionation in planktic foraminifera. With respect to the carbon isotopic fractionation, the importance of symbiont photosynthesis (e.g. Spero and DeNiro, 1987; Spero and Williams, 1989; Spero et al., 1991; Spero and Lea, 1993) and the effect of respiration (Spero and Lea, 1996) have well been documented. Subtle effects on the oxygen isotopic composition were noted but could not be explained

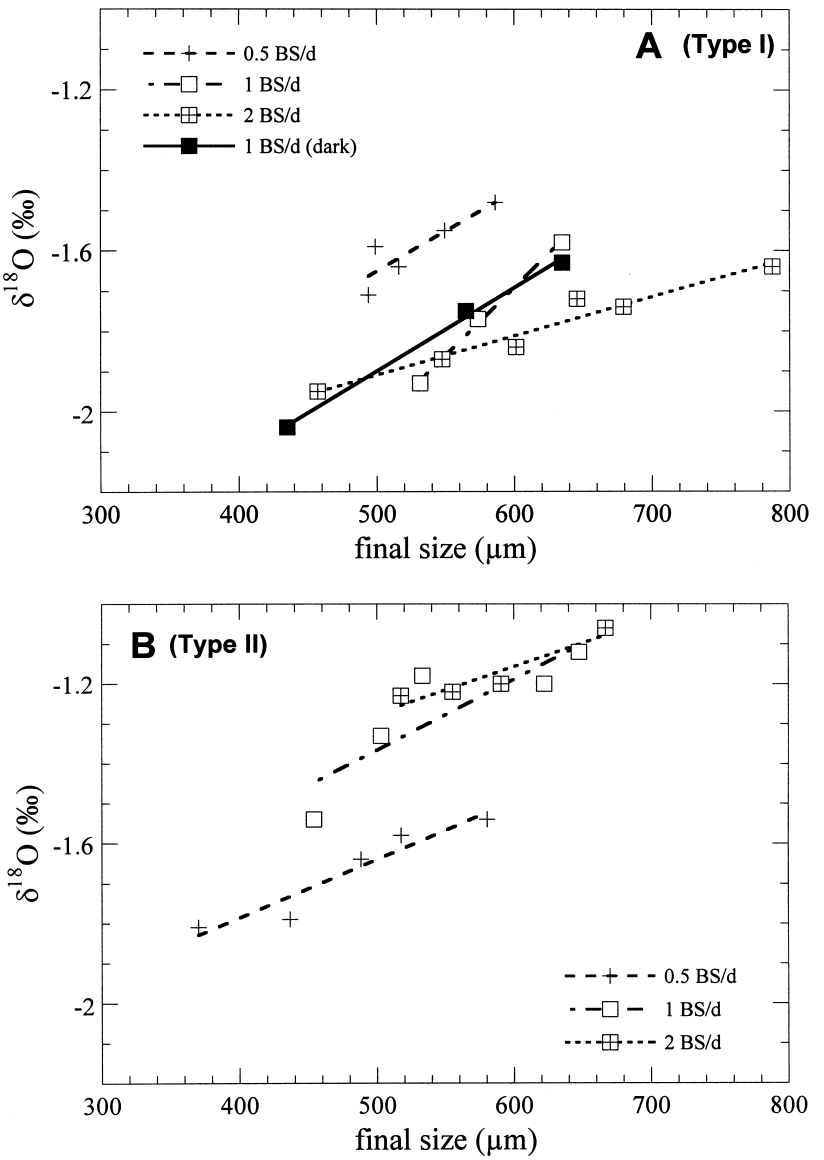

Fig. 6. The oxygen isotope ratio of *G. siphonifera* Type 1 (A) and Type II (B) that formed two chambers in culture at three different feeding schedules. All experiments were carried out under L1. Type I also formed chambers in continuous darkness (closed square) whereas Type II underwent gametogenesis or died in continuous darkness without forming chambers.

properly. In a recent study, Spero et al. (1997) and Bijma et al. (1998) have demonstrated that changes in the carbonate chemistry of the culture medium have a dramatic impact on both, the carbon and oxygen isotopic composition. In fact, these effects are so strong that they have to be considered when interpreting glacial isotope records (Lea et al., 1998). In view of these new results the isotopic fractionation mechanisms in foraminifera have to be reassessed.

Using pH- and oxygen-micro electrodes Jørgensen et al. (1985) and Rink et al. (1998) have elegantly shown that photosynthesis and respiration exert a major influence on the ambient carbonate

system. Using a numerical model, based on diffusion through a stagnant boundary layer and chemical conversion between the carbonate species, Wolf-Gladrow et al. (1999) were able to simulate changes in the ambient carbonate chemistry of foraminifera induced by photosynthesis, respiration and calcification. In a companion paper Zeebe et al. (1999) have demonstrated that the isotope effects observed by Spero et al. (1997) can largely be explained by kinetic fractionation. Apparently, changes in the ambient carbonate chemistry induced by the life processes have a first order, kinetic, impact on shell $\delta^{13}C$ and $\delta^{18}O$. The simultaneous shift in the $\delta^{13}C$ value of the ambient DIC by photosynthesis (enrichment with ^{13}C) or respiration (enrichment with ^{12}C) adds another level of shell $\delta^{13}C$ modification. Here, we first discuss the major differences in biology and growth characteristics between the two types under different culture treatments and then focus on possible mechanisms that could explain the observed isotopic differences.

4.1. Habitat differences between types I and II

The pigment scan (Fig. 2) demonstrates that symbionts of the two Types have the same photo-pigment composition but that Type I has less pigment per foram (Table 3). This is due to the lower number of symbionts and a lower quantity of pigment per symbiont (Faber et al., 1988). The chlorophyll *a* content found in this study for Type II is in close agreement with the 7.9 ng chlorophyll *a* determined for *G. siphonifera* (undifferentiated) of 317 μm (Bijma, 1986). The observation of a higher chlorophyll *a* content of Type II suggests that it is shade adapted and therefore occupies a deeper habitat than Type I. The preference for a certain light environment (depth habitat) may also be inferred from qualitative differences in pigment composition. The higher ratio of carotenoid (absorbing in the green part of the spectrum) to chlorophyll *a* for Type II may thus point towards a deeper habitat but this theory of complementary chromatic adaptation has been criticised (Dring, 1981). Contrarily, the differences with respect to the photopigment composition could also result from the different location of the symbionts in the two types. In Type I, they are dispersed between the spines whereas in Type II a substantial number of symbionts is found inside the shell or clustered along the spines (self shading). Consequently, the higher pigment content and higher ratio of carotenoid to chlorophyll *a* of the symbionts of Type II compared to Type I may be the result of adaptation to a locally different light regime.

Although a potential deeper life habitat for Type II is supported by the lower Mg/Ca ratio and the heavier stable oxygen isotope composition (indicating a 1.5–1.8ºC colder precipitation temperature, respectively), both types were collected in the same depth range between 3 and 6 m depth. In addition, the offset between types I and II with respect to their stable isotopic composition under identical laboratory conditions suggests that these differences are type specific and not induced by differences in habitat. We conclude therefore that the available evidence does not indicate depth habitat differences between the two types and that differences in pigment composition suggest that the potential photosynthetic rate of Type II is higher.

4.2. Growth experiments

In general, our laboratory cultures confirm the growth characteristics reported by Faber et al. (1989). However, while Faber et al. (1989) used all specimens that went into culture, i.e. their statistics includes specimens that died without undergoing gametogenesis, we restricted our analysis only to those that had undergone gametogenesis. Survival times are usually longer for specimens that die without reproduction than for specimens that undergo gametogenesis. Because the frequency of gametogenesis in culture is generally higher in Type II than in Type I (especially under high light), the survival times calculated by Faber et al. (1989) are biased towards longer survival times for Type I. As a result, they calculated an average survival time for Type I under high light (1 BS/d) which is 2.1× longer than for Type II. Under comparable conditions we find that the longevity of Type I is only 1.4× greater than for Type II. As a consequence, differences in chamber formation rates arise between the two studies. The rate of chamber formation for Type II is on the average 1.4× higher than for Type I (Table 2). On the contrary, under high light intensities and a high feeding schedule, Faber et al. (1989) calculate

a 2.5× higher chamber formation rate for Type II. Nevertheless, we can safely conclude that the calcification rate of Type II is much higher. However, it should be noted that the calculated calcification rates (in nmol/h) express an average calcification rate for the duration of the experiments and not the actual calcification rate during chamber formation.

4.3. Isotope experiments

In general, the $\delta^{13}C$ and $\delta^{18}O$ values of planktic foraminifers covary with shell size with larger shells typically becoming enriched in ^{13}C and ^{18}O (Kahn, 1977; Kahn and Williams, 1981; Curry and Matthews, 1981; Erez and Honjo, 1981; Bouvier-Soumagnac and Duplessy, 1985; Shackleton et al., 1985; Berger and Vincent, 1986; Deuser, 1987; Grossman, 1987; Oppo and Fairbanks, 1989; Ravelo and Fairbanks, 1992; Shuxi and Shackleton, 1990; Kroon and Darling, 1995). A heavier oxygen isotope composition implies a cooler (= deeper) habitat, whereas enrichment with ^{13}C suggests a shallow niche. The contradictory size-related trend observed in *G. siphonifera* demonstrates that interpretation of the isotope composition in terms of equilibrium precipitation is not straightforward. It has been shown that the carbon isotope composition of the shells of symbiont bearing foraminifera reflect the $\delta^{13}C$ of the DIC but that the signal is modulated by symbiont activity (Spero and DeNiro, 1987; Spero and Williams, 1988, 1989; Spero, 1992; Spero and Lea, 1993). These authors concluded that due to the higher affinity of the carbon fixing enzyme ribulose 1,5-biphosphate carboxylase-oxygenase (RUBISCO) for ^{12}C, the photosynthetic activity of the symbionts locally enriches the DIC with ^{13}C. As the number of symbionts increase with the host's ontogeny (Bijma, 1986; Spero and Parker, 1985), the carbon source for calcification will become progressively more enriched and hence result in a heavier signal. Under the constant light conditions in the laboratory this hypothesis may explain the ontogenetic trend towards heavier $\delta^{13}C$. In the field, however, the increase in the number of symbionts is offset by the migration towards a deeper habitat where the photosynthetically available radiation is increasingly reduced (Hemleben and Bijma, 1994). Hemleben and Bijma (1994) argued that the ontogenetic increase in oxygen and carbon isotopic composition are independent effects due to migration towards a deeper habitat (increasing $\delta^{18}O$) and an increase in the number of symbionts (increasing $\delta^{13}C$). They calculated that the gross assimilation rate increases notwithstanding the migration to a deeper habitat. However, the covariation between $\delta^{13}C$ and $\delta^{18}O$ observed under constant laboratory conditions (Figs. 4–6; Spero and Lea, 1996) demonstrates that other mechanisms must be invoked for the isotopic fractionation (especially regarding $\delta^{18}O$) and that an ontogenetic depth migration apparently plays little or no role.

There are also several arguments suggesting that the symbionts are not exclusively responsible for the observed trend of $\delta^{13}C$ with size:

(1) Although not as pronounced as in symbiotic species, non-symbiotic spinose species such as *Globigerina bulloides* d'Orbigny also show an ontogenetic carbon isotope trend (e.g. Kroon and Darling, 1995; Spero and Lea, 1996). In addition, specimens of Type I grown in continuous darkness show the same trend as specimens cultured under light, although the absolute $\delta^{13}C$ values are lower (Fig. 5A).

(2) Were photosynthetic enrichment the *only* vital effect, the carbon isotope composition of foraminiferal calcite should be heavier than $\delta^{13}C_{DIC}$ + 1‰ (Romanek et al., 1992). This was not observed in *G. sacculifer* (Spero and Lea, 1993) or *O. universa* (Spero, 1992) and adult chambers of types I and II are 0.7 and 0.3‰ more negative than calcite precipitated in equilibrium, respectively (Fig. 3). Kahn (1979) and Kroon and Darling (1995) also report a large negative offset for *G. siphonifera* and Pearson and Shackleton (1995) found the same phenomenon for *G. praesiphonifera*.

In summary, we have to invoke an additional mechanism that depletes the calcifying environment with ^{13}C or enriches it with ^{12}C. One likely candidate for ^{12}C enrichment is host respiration. Respired CO_2 is usually isotopically lighter than the carbon source compounds (e.g. McConnaughey and McRoy, 1979). If we assume that foraminiferal respiration releases ^{12}C-enriched CO_2 and thereby depletes the calcifying environment, this could partly explain the negative offset with respect to equilibrium calcite. It can be calculated that the CO_2 diffusion time scale is in the order of 100 seconds for a sphere with a radius of 300 μm and slow in comparison to chem-

ical conversion (Wolf-Gladrow et al., 1999). Thus a substantial part of the respired CO_2 may be chemically converted into bicarbonate but some will be assimilated by the symbionts. Therefore, in continuous darkness and under high feeding regimes, the most negative values are obtained. As more food is digested, more light respired CO_2 is released and as a consequence isotopically lighter shells are produced. This scenario explains the dependence of shell $\delta^{13}C$ of Type I on the feeding rate (Fig. 5A). Apparently, in *G. siphonifera* the impact of the decreasing $\delta^{13}C_{DIC}$ of the ambient environment due to respiration dominates the respiration driven decrease of the local pH (which would increase shell $\delta^{13}C$; Spero et al., 1997; Bijma et al., 1998).

The reason that a dependence on the feeding regime is not observed in Type II may be that a large number of symbionts is located inside the shell where they assimilate most of the respired CO_2 before it reaches the site of calcification and hence eliminate the effect of respiration. This also may explain why Type II is heavier than Type I. We then have to assume that this process dominates the impact of photosynthates on the ambient carbonate chemistry which would lead to more negative values. The increase of $\delta^{13}C$ with ontogeny could suggests that due to the increasing symbiont density the fraction of assimilated respired CO_2 increases as well. Consequently the shells become isotopically heavier with size. Apparently, the ontogenetic increase of the fraction of assimilated respired CO_2 is faster for Type II than for Type I.

At this point we may conclude that the $\delta^{13}C$ value of each newly formed chamber in *G. siphonifera* depends on the gross photosynthetic rate, the respiration rate and on the position of the symbionts with respect to shell surface. However, recent culture experiments where *G. bulloides* (Spero and Lea, 1996) and *O. universa* (unpubl. results) were fed brine shrimp nauplii differing in their carbon isotopic composition (-15 or -22‰) did not result in a large change of the isotopic composition of foraminiferal calcite in either species, suggesting that not more than 10% of the shell carbon originates from respired CO_2. Although, the experiments with *O. universa* are in agreement with the hypothesis that high symbiont densities may dampen or even completely dominate the effect of respired carbon, the results with the symbiont barren *G. bulloides* demonstrate that the impact of respiration is relatively small and that another mechanism must be invoked to explain a ^{13}C depletion or ^{12}C enrichment of the calcifying environment.

Substantial enrichment of the carbon isotope composition of the solid carbonate phase was observed during slow inorganic precipitation and the enrichment decreased with increasing precipitation rate (Turner, 1982). Kinetic fractionation during rapid biological precipitation, which is typically much faster than inorganic carbonate precipitation from the same water, may even lead to depletion (Mook, 1994). For example, McConnaughey (1989b) demonstrated that rapid skeletogenesis in corals causes substantial depletion in ^{13}C. Thus, the higher calcification rates calculated for Type II (Table 2) should give rise to more negative values than for Type I, which is the opposite of what we have observed. On the other hand, one could argue that a faster calcification rate would lower the ambient carbonate ion concentration (Wolf-Gladrow et al., 1999) and therefore produce a heavier carbon and oxygen isotopic shell composition (Spero et al., 1997; Bijma et al., 1998). It should further be noted that we did not determine in situ calcification rates but calculated an average value.

The fractionation effects during calcification in corals are thought to result from discrimination against ^{18}O and ^{13}C during hydration and hydroxylation of CO_2. These reaction steps are rate limiting, they may exhibit different kinetic isotope effects, and the balance between the two may change with pH (McConnaughey, 1989a). Because the equilibration of carbon and oxygen isotopes occurs simultaneously and mainly depend on the rates of diffusion and chemical conversion, this would produce a positive correlation between oxygen and carbon isotope ratios. Such a mechanism could explain the covariance between $\delta^{13}C$ and $\delta^{18}O$ observed during foraminiferal ontogeny and the linear correlation between the carbon and oxygen isotope ratios in *G. siphonifera* (Fig. 3). It is noteworthy that the slope (0.5) is much steeper than the range of 0.24–0.33 found for *O. universa* and *G. bulloides* (Spero et al., 1997; Bijma et al., 1998) and *G. sacculifer* and *G. ruber* (unpubl. results).

5. Conclusions

Although *G. siphonifera* types I and II occupy the same habitat and their empty shells are difficult to differentiate, they should be regarded as sister species (Huber et al., 1997). Important physiological differences (especially photopigment composition, symbiont position and probably calcification rate) give rise to very different isotopic compositions. Because $\delta^{13}C$ of Type I gets lighter at higher feeding frequency but Type II remains unaffected, the $\delta^{13}C$ difference between the two types could be used as a proxy for productivity. However, due to the similarity of the empty shells this species is not very suitable for routine paleoceanographic or -climatic investigations.

The processes involved in stable isotope fractionation are complex and not yet completely understood. Although significant uncertainties remain with regard to some aspects, a model combining the impact of the life processes on the ambient carbonate system with their effect on the ambient $\delta^{13}C_{DIC}$ presents a challenging way to explain disequilibrium fractionation and the observed ontogenetic trends in both oxygen and carbon isotopes in planktic foraminifers. An important implication is that, because vital effects are mediated by the carbonate system, changes in the carbonate chemistry of the ocean such as during climatic oscillations (Sanyal et al., 1995), may have a significant impact on the stable isotope composition of foraminiferal shells which in turn has important implications for paleoceanographic interpretation of these chemical proxies (Lea et al., 1998).

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