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Geochemical investigation of gametogenic calcite addition in the planktonic foraminifera *Orbulina universa*

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ABSTRACT

Several species of planktonic foraminifera precipitate a final layer of calcite onto the shell surface immediately prior to gamete release at the end of the foraminifera life cycle. Here, we present the results of carbon-13, oxygen-18 and thermal labeling experiments conducted under high (HL) and low light (LL) regimes that vary symbiont photosynthetic activity. Mean experimental group data show that gametogenic (gam) calcite contributes between 4–17% and 14–20% to final shell mass for high and low light experiments respectively. These data indicate that past studies of gam calcite addition have overestimated the amount of gam calcite on foraminifera shells by ~30–55%. Calculations indicate that the mass of gam calcite added to the *O. universa* shell, 4.2±2.0 μg and 4.0±2.4 μg, is constant in the HL and LL groups respectively. We propose that the production of gam calcite may be the result of the discharge of a relatively constant-volume cytoplasmic pool of either Ca^{2+} or alkalinity (carbon pool) that increases the calcite saturation state in the microenvironment adjacent to the foraminifera shell just prior to gamete formation and release. Results from these experiments indicate that the geochemistry of thin-walled *O. universa* from deep sea sediments is composed of >80% ontogenetic calcite that was precipitated in the primary, near surface habitat of this species.

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1. Introduction

The geochemical composition of the calcite tests of fossil planktonic foraminifera from deep sea sediments is commonly used to reconstruct paleoenvironmental conditions. Laboratory and field studies with modern/living foraminifera demonstrate that shell $\delta^{18}O$ and $\delta^{13}C$ are controlled by a variety of environmental and ecological parameters such as the carbon and oxygen isotopic composition of seawater, temperature, pH, seasonal and depth habitat preferences and a variety of other biologically-related processes such as symbiont photosynthesis, respiration and food geochemistry (Bemis et al., 1998; Bemis et al., 2000; Bijma and Hemleben, 1994; Bijma et al., 1999; Sautter and Thunell, 1991; Spero, 1992; Spero et al., 1997; Spero and Lea, 1993; Spero and Lea, 1996; Uhle et al., 1999). Constraining sources of intraspecific isotope variability and quantifying isotopic disequilibria among different species is an essential element in accurate reconstructions of past water column hydrography and climatic conditions.

A number of species of foraminifera (e.g. *Globigerinoides sacculifer*, *G. conglobatus*, *Orbulina universa*, *Neogloboquadrina pachyderma*, *N. dutertrei* and *Globorotalia* spp.) are known to deposit an additional calcite crust of variable thickness on the outer surface of their shells (Bé, 1980; Bé et al., 1983; Bé et al., 1973; Caron et al., 1990; Hemleben et al., 1985; Srinivasan and Kennett, 1974). In species such as *G. sacculifer*, *G. conglobatus*, and *O. universa*, this secondary calcite crust is associated with the release of gametes at the end of the life cycle and is commonly called gametogenic or 'gam' calcite. In *G. sacculifer*, gametes are usually released within 24 h of gam calcite addition (Bé et al., 1983). In other species, such as *N. dutertrei* or the *Globorotalid* species, a calcite crust may be deposited in

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response to a reduction in temperature as the organism descends through the water column (Hemleben and Spindler, 1983; Hemleben et al., 1985; Srinivasan and Kennett, 1974). Although this type of crusting is thought to be different from gam calcite addition, it also occurs near the end of the foraminifera life cycle. In this paper, we define gam calcite as the layer of calcite that is rapidly deposited on the outside surface of a shell during the transition from normal ontogeny to the meiotic reproductive phase of some species that occurs during the last 24 h of the organism's life cycle (Bé, 1980; Bé et al., 1983).

The physiological and morphological events leading up to gam calcite precipitation have been described previously (Bé, 1980; Bé et al., 1983; Spero, 1988). In a species such as *O. universa*, the lifespan is approximately one lunar cycle (Hemleben and Bijma, 1994). Normal ontogenetic calcification encompasses the precipitation of lightly calcified chambers in a trochospiral whorl and the subsequent secretion and continuous thickening of a large terminal spherical chamber around the trochospiral shell, 4–7 days prior to gam calcification (Spero, 1988). The gam calcification event is immediately preceded by a progressive shortening of the foraminifera spines from a typical ontogenetic length of ~2.5 mm to <10 μm. A detailed ultrastructural study of *G. sacculifer* indicates the cellular structure undergoes a fundamental reorganization in preparation for gametogenesis, as the gam calcification phase ends (Bé et al., 1983). Observations suggest *O. universa* follows a similar reorganization pattern (Spero, 1986), and within 24 h after the initiation of spine shortening, *O. universa* releases all of its gametes leaving behind an empty shell that sinks through the water column and becomes part of the sedimentary record.

Calcification in *O. universa* can occur under varying environmental and physiological conditions (e.g. symbiont photosynthetic rates) as a foraminifer matures and slowly floats/sinks across different depths in its photic zone habitat range. Ontogenetic calcification occurs throughout the *O. universa* lifespan, whereas gam calcification occurs at the very end of its life cycle over a short period of time. Because ontogenetic calcification is constrained to the photic zone in order to accommodate symbiont photosynthesis, and gam calcification is only depth constrained by the density of the sphere and lipid content of the encapsulated cytoplasm during ultrastructural reorganization, the geochemistry of these two calcification phases could reflect different environmental conditions. Lohmann (1995) presented a mass balance based model in an attempt to distinguish between the chemistries of ontogenetic and gam calcite in fossil assemblages of *G. sacculifer*, thereby demonstrating some of the difficulties in interpreting geochemical data with respect to primary habitat environment. This research highlights the potential influence of gam calcite on the acquisition of geochemical signatures via purely surface processes as opposed to a mixture of surface and deeper water column influences.

To interpret fossil geochemical data from species that produce gametogenic calcite it is important to quantify the amount of gam calcite that a foraminifera produces at the end of its life cycle. For instance, Bé (1980) calculated that core top *G. sacculifer* collected from sediment near Barbados were on average 7.4 μg more massive than similarly sized shells obtained in plankton tows. He concluded that this mass difference was due to gam calcite addition. In this study we present the results of a series of carbon and oxygen isotope labeling experiments with living *O. universa* that quantifies the amount of gametogenic calcite added to a shell under different environmental conditions. These data provide a starting point for understanding the physiological mechanism responsible for gam calcite addition and offer insight into potential ways of addressing the impact of the geochemistry of gam calcite for environmental reconstructions.

2. Materials and methods

Laboratory experiments were conducted at the Wrigley Marine Science Center (WMSC), Santa Catalina Island, California during the summers of 1993, through 1995. Trochospiral stage, *O. universa* were hand collected at 1–3 m depth by scuba divers approximately 2 km NNE of WMSC (33°23′N, 118°26′W). During each collection, surface water temperature and salinity were measured and water samples were taken for analysis of seawater $\delta^{18}O$ and $\delta^{13}C$ of dissolved inorganic carbon ($\delta^{13}C_{DIC}$).

Experimental procedures follow previously established culture protocols for living planktonic foraminifera (Bemis et al., 1998). After collection, each specimen was visually inspected and measured with an inverted microscope, placed in a 120 ml acid-washed borosilicate jar containing 0.8 μm pore size filtered seawater, and fed a single one-day-old *Artemia* nauplius (San Francisco Bay Strain, Artemia Reference Center #1157 $\delta^{13}C_{artemia} = -21.5‰$ (Spero et al., 1993)) on the collection day and again every third day. Jars were filled to the top and capped to minimize gas exchange with laboratory air, then maintained at a constant temperature of 22±0.2 °C. Experiments were conducted at two light levels — high light (400–700 μmol photons m^{-2} s^{-1}) which is above the P_{max} level of 365 μmol photons m^{-2} s^{-1} and low light (26–30 μmol photons m^{-2} s^{-1}) which is below the foraminiferal-symbiont compensation light level (Rink et al., 1998; Spero and Parker, 1985).

Carbon-13 and oxygen-18 enriched seawaters were prepared by the quantitative addition of 99.1% $Na_2^{13}CO_3$ or 1.5% ^{18}O-enriched water to seawater. Three isotopically-labeled solutions were prepared for the experiments, a ^{13}C spike, an ^{18}O spike, and a combined ^{13}C–^{18}O spike. A fourth experiment incorporated a 15 °C thermal spike, which has a nominal effect on shell $\delta^{13}C$ in *O. universa* (Bemis et al., 2000), and primarily influences the $\delta^{18}O$ of precipitated calcite. Control specimens were grown entirely in seawater or spiked solutions to produce spiked and seawater endmember (EM) values

Table 1
Isotopic endmembers (EM) for experimental groups

Experiment	Ambient EM values		Spike EM values	
	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)
HL ^{13}C spike	−2.00±0.09	2.98±0.19	−1.62±0.09	38.38±0.47
LL ^{13}C spike	−1.62±0.14	1.55±0.11	−1.62±0.14	38.38±0.27
HL ^{18}O spike	−2.10±0.11	3.54±0.10	9.38 [a]	1.48±0.10
LL ^{18}O spike	−1.76±0.06	1.48±0.15	9.38 [a]	1.48±0.15
HL double spike	−2.00±0.09	2.98±0.19	30.20±0.41	9.87±0.50
LL double spike	−1.62±0.14	1.55±0.11	30.20±0.62	9.87±0.18
HL thermal spike	−2.10±0.11	3.54±0.10	−0.23 [a]	1.48±0.10
LL thermal spike	−1.76±0.06	1.48±0.15	−0.23 [a]	1.48±0.15

[a] EM values computed from equations of Bemis et al (1998).

Table 2
Pre-gam vs. gam *O. universa*, at 22 °C under HL and LL

Experiment	*n*	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)
HL gam	6	−2.01±0.08	3.00±0.16
HL pre-gam	6	−1.99±0.10	2.98±0.22
LL gam	6	−1.61±0.11	1.55±0.12
LL pre-gam	7	−1.64±0.16	1.55±0.11

to use in our calculations of gam calcite addition (see Eq. (2)). The spiked EM values for the ^{18}O and thermal experiments were computed using the equations of Bemis et al. (1998). During our three field seasons, the enriched $\delta^{13}C_{DIC}$ spike used for the ^{13}C spike and double spike experiments in years 2 and 3 differed from year 1. EM $\delta^{13}C$ values for *O. universa* from these latter years are not available. However, because $\delta^{13}C_{DIC}$ measurements were made on the spiked water and the isotopic composition of *O. universa* faithfully records changes in seawater $\delta^{13}C_{DIC}$ with a 1:1 relationship (Spero, 1992), we computed the spiked ^{13}C EM shell values by adding the $\delta^{13}C_{DIC}$ difference between year 1 and subsequent year $\delta^{13}C_{DIC}$ to the year 1 shell EM values. The $\delta^{18}O_{water}$ spikes were identical in each field season and therefore required no offset correction. Table 1 summarizes the experimental EM data. Because the symbionts are either digested or lost prior to gam calcite addition (Bé et al., 1983), thereby eliminating the effect of symbiont photosynthesis on gam calcite oxygen isotope geochemistry, we compute the gam calcite EM for the thermal experiment using the Bemis et al. (1998) low light relationship for both low and high light gam calcite experiments, together with a measured ambient $\delta^{18}O_{water}$ value of −0.25‰.

Once the spines had shortened to <1 mm, an indication that the gam calcite addition phase was imminent (Bé, 1980; Spero, 1988), several specimens were removed from culture to analyze the mass and stable isotopic composition of ontogenetic, or pre-gametogenic (pre-gam) calcite. Some specimens were allowed to undergo gametogenesis under ambient conditions (under both high and low light) to determine the ambient gametogenic isotopic endmembers (Table 1). At spine shortening, a subset of the specimens were transferred into jars containing one of the isotopic spike combinations, or into a water bath at 15 °C, to label the gam calcite with a unique and known isotopic composition. Following the release of gametes, the empty tests were rinsed in deionized water and archived for later processing.

Prior to stable isotope analyses, individual archived foraminifera were weighed on an ultramicrobalance and measured with a binocular microscope. The shells for carbon and oxygen analysis were roasted at 365 °C for 1 h *in vacuo* and then analyzed individually by an Isocarb autocarbonate device attached to a Fisons Optima isotope ratio mass spectrometer (IRMS). Data are presented as δ values in per mil (‰) notation relative to the Vienna Pee Dee Belemnite (VPDB) standard where

$$\delta = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000 \quad (1)$$

and R_{sample} or $R_{standard}$ refer to either $^{13}C/^{12}C$ or $^{18}O/^{16}O$ ratios of the sample or standard respectively. The analytical precision for the *O. universa* analyses is ±0.03‰ and ±0.09‰ for $\delta^{13}C$ and $\delta^{18}O$ respectively, as determined by replicate analyses of a Carrera marble standard in the same mass range as the foraminifera.

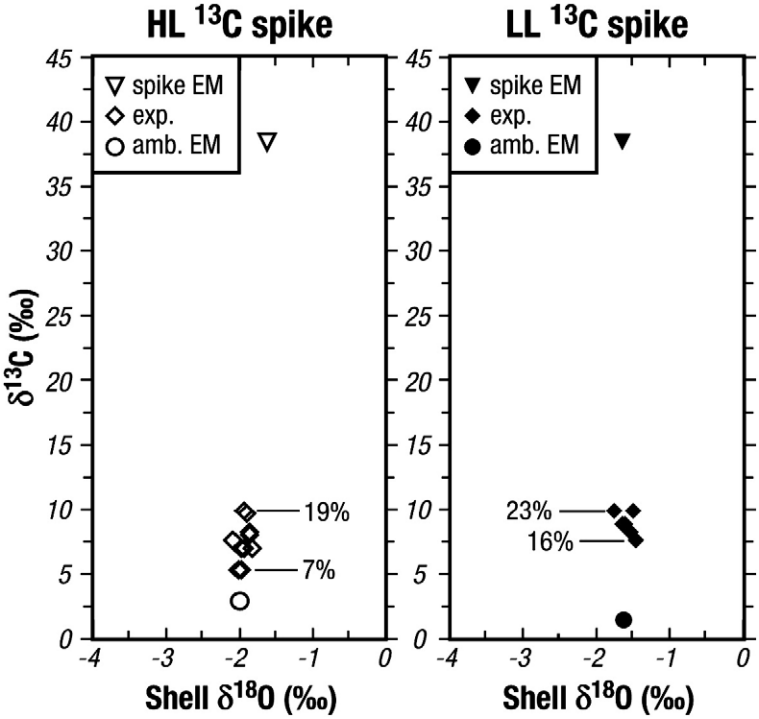

Fig. 1. High light and low light experiments with a $\delta^{13}C$ spike.

The percent gam calcite addition for each specimen was calculated using a simple mass balance relationship:

$$\delta_{\text{shell}}\,(‰) = Ax + B(1-x) \quad (2)$$

where x = fraction of gam calcite relative to total shell calcite, A = gam calcite δ value (spiked EM) and B = ontogenetic calcite δ value (ambient EM). The mass addition of gam calcite for an individual was calculated from the percent gam calcite addition.

A suite of the experimental culture and seawater spikes were collected for analysis at the initiation of each experiment. Water samples were not collected at the conclusion of the experiments, but data from prior field seasons and experiments have shown that the sealed vial technique we use for our foraminifera experiments maintains the $\delta^{18}O_{water}$ at constant values (Bemis et al., 1998) and shows only nominal change in the $\delta^{13}C_{DIC}$ (<0.2‰) (Spero et al., 1997) during an experiment. For $\delta^{13}C_{DIC}$, water samples were processed off-line by injecting 5 ml of water onto H_3PO_4 *in vacuo*. The resulting CO_2 was purified, collected cryogenically and analyzed with a Fisons Optima IRMS. Data are reported relative to VPDB with a long-term precision of ±0.03‰ based on repeat analyses of a prepared, in-house DIC standard. $\delta^{18}O_{water}$ was determined with an automated CO_2–H_2O equilibrator that was attached to a Finnigan MAT 251 IRMS. Values are reported

Table 3
Orbulina universa shell diameter, thickness, mass, $\delta^{18}O$ and $\delta^{13}C$ values, % and mass of gam calcite (GC) addition

Experiment	HL/LL	Diameter (μm)	Mass (μg)	Thickness [a] (μm)	Shell $\delta^{18}O$ (‰)	Shell $\delta^{13}C$ (‰)	% GC ^{13}C spike	Mass (μg) GC ^{13}C spike	% GC ^{18}O spike	Mass (μg) GC ^{18}O spike
^{13}C spike	HL	505	34	18.2	−1.99	5.29	7	2		
	HL	531	36	17.3	−1.97	5.27	7	3		
	HL	569	38	15.7	−1.96	6.82	11	4		
	HL	532	36	17.2	−1.83	6.79	11	4		
	HL	595	45	17.1	−1.92	6.86	11	5		
	HL	575	40	16.3	−2.08	7.42	13	5		
	HL	477	22	13.0	−1.84	7.83	14	3		
	HL	535	33	15.5	−1.86	8.08	15	5		
	HL	532	42	20.3	−1.87	9.52	18	8		
	HL	520	34	17.0	−1.91	9.73	19	6		
	LL	680	36	10.2	−1.44	7.55	16	6		
	LL	555	28	12.1	−1.53	8.07	18	5		
	LL	496	21	11.4	−1.62	8.81	20	4		
	LL	606	29	10.4	−1.59	8.86	20	6		
	LL	520	14	6.8	−1.48	9.80	22	3		
	LL	606	25	8.9	−1.74	9.86	23	6		
^{18}O spike	HL	648	51	16.2	−1.77	3.34			3	2
	HL	665	53	16.0	−1.80	3.46			3	1
	HL	620	46	16.0	−1.51	2.89			5	2
	HL	632	47	15.7	−1.56	3.15			5	2
	LL	550 [b]	31	13.7	−0.11	1.78			15	5
	LL	585 [b]	24	9.2	0.21	1.78			18	4
	LL	607 [b]	26	9.3	0.28	1.56			18	5
	LL	566 [b]	31	12.9	0.56	1.62			21	7
Double spike	HL	569	42	17.5	−0.55	3.29	4	2	5	2
	HL	588	40	15.5	−1.00	3.29	4	1	3	1
	HL	588	61	24.3	1.63	3.35	5	3	11	7
	HL	532	48	23.5	0.07	3.36	5	2	6	3
	HL	**771**	**97**	**21.9**	**3.18**	**3.92**	**13**	**13**	**16**	**15**
	HL	532	33	15.7	2.28	3.92	14	5	13	4
	HL	**881**	**80**	**13.5**	**3.83**	**4.08**	**15**	**12**	**18**	**14**
	LL	441	18	12.4	0.18	1.79	3	1	6	1
	LL	495	14	7.5	1.60	2.02	6	1	10	1
	LL	532	25	11.7	2.03	2.05	6	1	11	3
	LL	496	23	12.5	1.78	2.12	7	2	11	2
	LL	496	29	16.0	3.03	2.60	13	4	15	4
	LL	441	18	12.4	4.18	3.08	18	3	18	3
	LL	441	20	13.9	4.51	3.11	19	4	19	4
	LL	514	18	9.0	6.25	3.58	24	4	25	4
	LL	514	18	9.0	8.13	3.98	29	5	31	6
Thermal spike	HL	629	48	16.2	−1.86	3.45			13	6
	HL	646	52	16.7	−1.71	3.08			21	11
	HL	**970**	**41**	**5.6**	**−1.70**	**3.60**			**21**	**9**
	HL	**970**	**27**	**3.7**	**−1.62**	**3.66**			**26**	**7**
	HL	**970**	**29**	**4.0**	**−1.52**	**3.83**			**31**	**9**
	LL	584 [b]	32	12.5	−1.68	1.67			5	2
	LL	572 [b]	32	13.0	−1.56	1.70			13	4
	LL	600 [b]	32	11.8	−1.31	1.87			29	9

Bolded specimens were not included in statistics because of their unusually large sphere diameters.
[a] Shell thickness computed using technique of Billups and Spero (1995).
[b] Two or more specimens combined; mean mass/diameter given.

Table 4
Experiment summary (Mean data are ±1σ)

Experiment	*n*	% GC addition range	Mean GC increase % shell mass	GC mass range (μg)	Mean mass increase (μg)
HL ^{13}C spike	10	7–19	12.6±4.1	2–8	4.5±1.7
LL ^{13}C spike	6	16–23	19.8±2.6	3–6	5.0±1.3
HL ^{18}O spike	4	3–5	3.8±1.3	1–2	1.9±0.5
LL ^{18}O spike	4	15–21	17.9±2.5	4–6	5.0±1.0
HL double spike					
^{13}C component	5	4–14	6.4±4.3[a]	1–5	2.6±1.5[a]
^{18}O component	5	3–13	7.6±4.2[a]	1–7	3.4±2.3[a]
LL double spike					
^{13}C component	9	3–29	13.9±9.1	1–5	2.8±1.6
^{18}O component	9	6–31	16.2±7.9	1–6	3.1±1.6
HL thermal spike	2	13–21	17.0±5.7[a]	6–11	8.5±3.5[a]
LL thermal spike	3	5–29	15.9±12.3	2–9	5.0±3.9

[a] Averages do not include bolded specimens in Table 3.

relative to VSMOW with a long-term precision of ±0.05‰ based on repeat analyses of three laboratory standards.

3. Results

The mean δ^{13}C and δ^{18}O values of *O. universa* maintained in ambient seawater and under high light are enriched by 1.44‰ and depleted by 0.37‰ respectively compared to shells grown in low light (Table 1). These shifts in isotopic composition are consistent with previously reported effects of symbiont photosynthesis for this species (Bemis et al., 1998). Specimens removed from culture at spine shortening (pre-gam) and specimens that were allowed to add gam calcite under ambient seawater conditions have δ^{13}C and δ^{18}O values that are indistinguishable within a light group (Table 2). These data show that differences between the isotopic geochemistry of gam and ontogenetic calcite, when deposited under identical conditions, are negligible and not a factor when interpreting stable isotope data in the fossil record.

In the first labeling experiment, pre-gam specimens were transferred from ambient seawater into ^{13}C-enriched water upon spine shortening to label the gam calcite (Fig. 1). Control *O. universa* were also grown entirely in ambient or ^{13}C-enriched seawater to establish isotopic EM values (Table 1). These shells yielded ambient EM δ^{13}C values of 2.98 and 1.55‰ for high and low light respectively, and a ^{13}C-spiked EM value of 38.38‰. High light shells with ^{13}C-labeled gam calcite have δ^{13}C values that range from 5.27 to 9.73‰, whereas low light *O. universa* ranged from 7.55 to 9.86‰ (Table 3). The results of mass balance calculations that use pre-gametogenic calcite (ambient) and

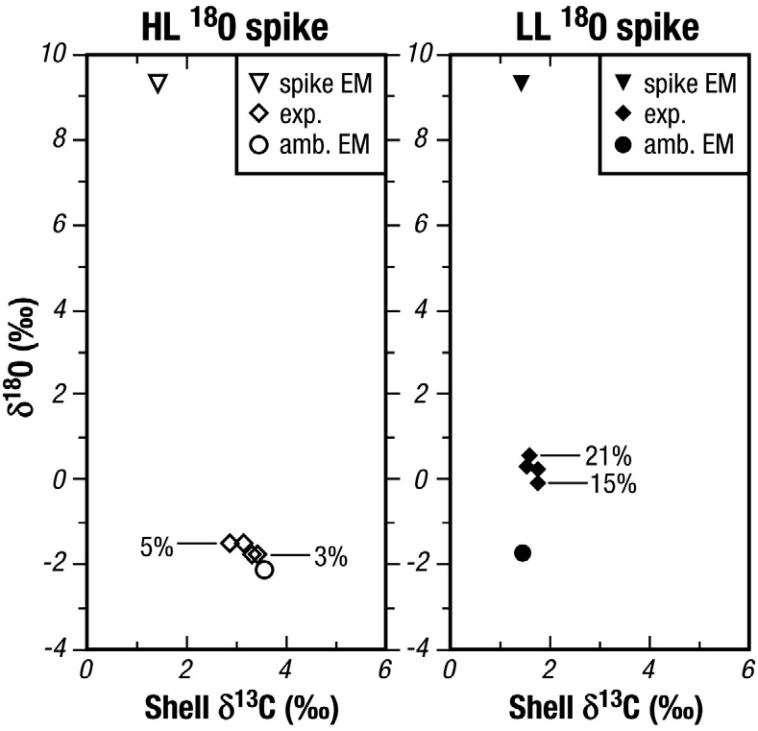

Fig. 2. High light and low light experiments with a δ^{18}O spike.

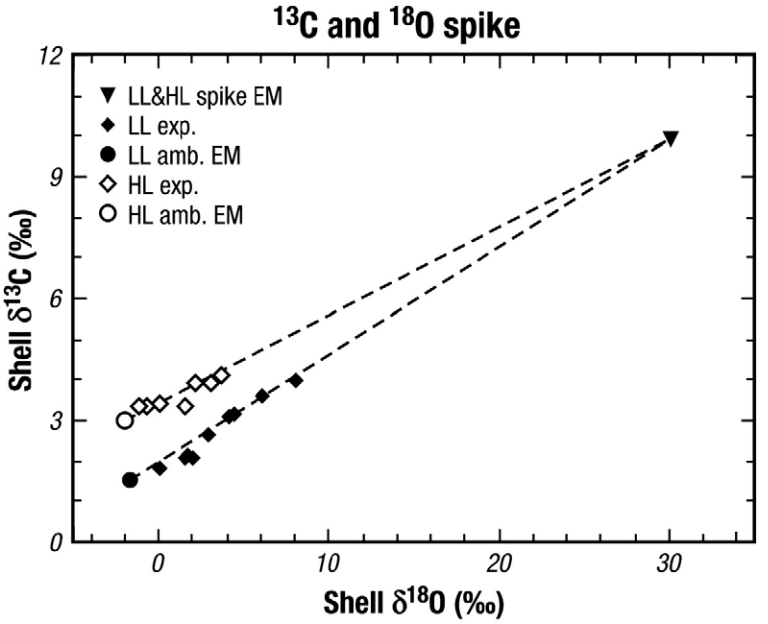

Fig. 3. High light and low light experiments with combined $\delta^{13}C$ and $\delta^{18}O$ spikes.

gam calcite (spiked) EM values, yield a gam calcite addition of 7 to 19% ($\bar{x}$±1 S.D.=12.6±4.1%) and 16 to 23% ($\bar{x}$=19.8±2.6%) for high and low light experiments respectively (Fig. 5 and Table 4).

In a second experiment, gam calcite was labeled with an ^{18}O-enriched seawater spike (Fig. 2). Here, the ambient EM values, determined from specimens grown entirely in filtered seawater, had $\delta^{18}O$ values of −2.10 and −1.76‰ for high and low light respectively. The $\delta^{18}O_{water}$ of the spiked solution was 10.80‰, which yields a calculated spiked EM *O. universa* $\delta^{18}O_{shell}$ value of 9.38‰ when precipitated at 22 °C (Bemis et al., 1998). *Orbulina universa* with ^{18}O-spiked gam calcite had $\delta^{18}O$ values ranging from −1.80 to −1.51‰ for the high light experiment and −0.11 to 0.56‰ for the low light experiment (Table 3). These values yield a gam calcite addition of 3

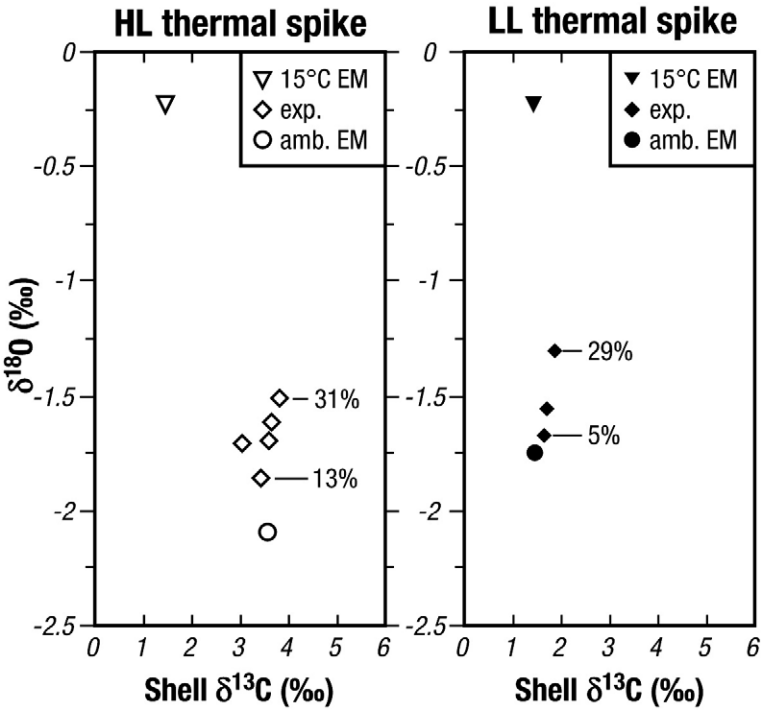

Fig. 4. High light and low light experiments with ontogenetic calcite precipitated at 22 °C and gam calcite precipitated in a 15 °C thermal spike.

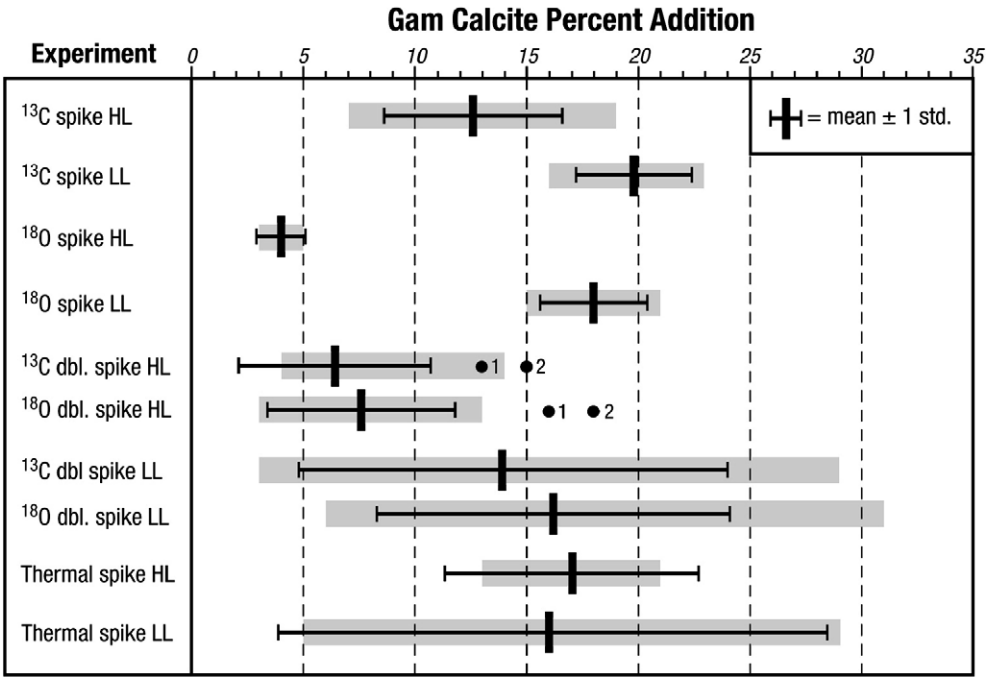

Fig. 5. Percentage gam calcite addition by experiment. Vertical black bars and horizontal lines are the experimental means ± 1σ. The shaded region encompasses the full range of experimental values. The two solid circles are data from massive specimens, and were not included in means. HL = high light experiment, LL = low light experiment.

to 5% ($\bar{x}$ ± 1 S.D. = 3.8 ± 1.3%) and 15 to 21% ($\bar{x}$ = 17.9 ± 2.5%) for the high and low light experiments respectively (Fig. 5 and Table 4).

A third experiment utilized a double spike of ^{13}C and ^{18}O, thereby allowing us to make two measurements of gam calcite addition on the same specimen (Fig. 3). In this experiment, the respective $\delta^{18}O$ and $\delta^{13}C$ ambient EM values were −1.62‰ and 1.55‰ for low light and −2.00‰ and 2.98‰ for high light. The measured spiked EM values were 30.20‰ for $\delta^{18}O$ and 9.87‰ for $\delta^{13}C$. The cultured foraminifera produced $\delta^{13}C$ values ranging from 3.29 to 4.08‰ in high light and 1.79 to 3.98‰ in low light whereas $\delta^{18}O$ values ranged from −1.00 to 3.83‰ in high light and 0.18 to 8.13‰ in low light (Table 3). These isotopic values yielded gam calcite additions that range from 4 to 14% ($\bar{x}$ ± 1 S.D. = 6.4 ± 4.3%) and 3 to 13% ($\bar{x}$ = 7.6 ± 4.2%) for the high light ^{13}C and ^{18}O spikes respectively, and 3 to 29% ($\bar{x}$ = 13.9 ± 9.1%) and 6 to 31% ($\bar{x}$ = 16.2 ± 7.9%) for the low light spikes (Fig. 5 and Table 4).

In a final experiment, specimens were transferred from ambient seawater at 22 °C into seawater at 15 °C once spine shortening was detected (Fig. 4). This experiment simulates the addition of gam calcite in a hypothetical thermocline on top of ontogenetic calcite that precipitated in a warmer mixed layer. We used ambient 22 °C $\delta^{18}O$ EM values determined in our second experiment (Table 1) and calculated the 15 °C thermal EM, $\delta^{18}O_{shell}$ = −0.23‰, using the low light equation of Bemis et al. (1998) with a measured $\delta^{18}O_{water}$ = −0.25‰. The $\delta^{18}O$ values of specimens with gam calcite added at 15 °C ranged between −1.86 to −1.52‰ and −1.68 to −1.31‰ for the high and low light experiments respectively (Table 3). This yielded a calculated gam calcite addition that ranged between 13 to 21% ($\bar{x}$ = 17.0 ± 5.7%) and 5 to 29% ($\bar{x}$ = 15.9 ± 12.3%) for the high and low light experiments respectively (Fig. 5 and Table 4).

4. Discussion

The mean range of gam calcite addition to the *O. universa* sphere, 3.8–19.8%, is considerably lower than estimates from other researchers. For instance, Erez and Honjo (1981) calculated a gam calcite addition of 36% to the final shell mass of *O. universa* by comparing plankton and sediment trap specimens. Similarly, Bé (1980) suggested an addition of 28% gam calcite to the test of the symbiont symbiont-bearing species *G. sacculifer*, based on comparison between surface sediment and plankton tow shell masses, and Caron et al. (1990) concluded gam calcite contributed 180–250% to *O. universa* shells when sedimentary shell masses were compared with shell weights from pre-gam specimens that had died in the laboratory.

A problem exists with these and other such computations. The determination of pre-gam shell mass in each of these cases was based on mass and size measurements of plankton tow specimens or 'dead' specimens in the laboratory without confirmation that the final chamber in the trochospiral whorl (*G. sacculifer*) or sphere (*O. universa*) was completely calcified with a 'pre-gam' mass. For example, the final chamber of a *G. sacculifer* specimen with a shell size of 500 μm can weigh >20 μg after chamber calcification is complete (Spero and Lea, 1993), and the *O. universa* sphere continues to add ontogenetic calcite until just before spine shortening (Lea et al., 1995; Spero, 1988). Thus, it would be easy to underestimate the pre-gam shell mass and overestimate the % gam calcite addition to a shell if the last *G. sacculifer* chamber or the *O. universa* sphere was not fully calcified.

A second complication exists when comparing living *O. universa* (e.g. tow or laboratory-grown shells) with shells from sediment assemblages. It is well documented from the

tropical Atlantic and Indian Ocean that *O. universa* occurs as both thick and thin thin-walled varieties (Bé et al., 1973; Billups and Spero, 1995; Deuser, 1987). Scanning electron microscopic measurements show the thin thin-walled variety ranges from ~12–25 μm in thickness whereas the thicker variety exceeds 40 μm. Thus, a simple comparison of average shell masses from the sediment with plankton tow specimens or *O. universa* that died in the laboratory could lead to an overestimation of the contribution of gam calcite to the final shell weight.

Analysis of the mean experimental shell diameters and shell masses from the high light and low light experiments here, indicates the sphere dimensions are similar, with diameters of 544±69 μm and 568±48 μm respectively. In contrast, HL and LL shell masses differed considerably, 42.0±8.9 μg (n=21) and 24.7±6.4 μg (n=22) respectively, most likely reflecting the impact of symbiont photosynthesis on daytime calcification (Lea et al., 1995). Utilizing an equation developed to compute *O. universa* shell thickness from shell mass and diameter (Billups and Spero, 1995), we obtain average shell thicknesses for HL and LL *O. universa* of 17.3 and 11.2 μm respectively (Table 3). These shell thickness calculations are comparable with shell thicknesses computed for 72 individual *O. universa* obtained from a nearby San Pedro Basin box core at 896 m water depth, 14.4±3.1 μm, using a mean population diameter of 469±76 μm and mean shell mass of 23.4±8.6 μg. The experimental shell thickness also falls within the observed range of the thin-walled variety of *O. universa* from the tropical Atlantic and Indian Ocean (Bé et al., 1973). Importantly, these shell thicknesses include the contribution of gam calcite to the outer shell surface.

Although the difference between thick-walled and thin-walled *O. universa* in the tropics could be due to the production of massive shells with subsequent dissolution of some spheres, or the addition of gam calcite on some shells and not others, we suggest an alternative explanation for these two morphotypes. It is well documented that many species of planktonic foraminifera display multiple genotypes and demonstrate cryptic speciation that complicates taxonomic identification of morphologically similar species or separation of distinct species from species 'complexes' (Huber et al., 1997; Kucera and Darling, 2002). Genetic analyses of the small subunit ribosomal RNA of *O. universa* shows the species is made up of at least three genotypes in the tropical Atlantic and elsewhere (Darling et al., 1999; Darling et al., 1997; Vargas et al., 1999). We suggest that such cryptic speciation could account for the bimodal thickness variation observed in sediment assemblages of *O. universa*. In this regard, we note that thick-walled *O. universa* have not been observed in either the laboratory or sediment assemblages of the Southern California Borderland, a region from which only one genotype of *O. universa* has been identified. However, in the tropical Atlantic, this Southern California/Santa Barbara Basin genotype has been found in association with two other *O. universa* genotypes (Kucera and Darling, 2002). If thickness differences can be attributed to different genotypes, we cannot exclude the possibility that the amount of gam calcite added to a sphere from a different genotype could differ from the results reported here.

It is clear why gam calcite is commonly observed on many shells collected from the sediment but is almost never found on specimens collected in plankton tows (Bé, 1980; Ericson and Wollin, 1968; Hemleben et al., 1985). Although some researchers have suggested that gam calcite may be added at depths of several hundred meters (Bé, 1980; Duplessy et al., 1981), others propose a shallower addition that is dependent on the depth of the mixed layer (Deuser, 1987). Using $\delta^{18}O$ values from individual fossil *G. sacculifer*, Lohmann (1995) demonstrated that most gam calcite addition in this species occurs within the photic zone/thermocline, just below the mixed layer. It is likely that the majority of *O. universa* and other symbiont-bearing planktonic foraminifera add gam calcite and reproduce in this depth region because the small gametes released during gametogenesis are aposymbiotic and must reacquire their photosynthetic symbionts to survive (Bé et al., 1983).

However, despite the limitation that symbiotic foraminifera must reproduce within the photic zone in order to maintain a viable population, Lohmann (1995) points out that some proportion of a population does add gam calcite at much deeper depths (300–500 m) and reasons that these shells represent individuals who missed their population reproduction depth and no longer contribute gametes/genes for the continuity of the population in the photic zone. Regardless of the surface dwelling species studied, individuals who miss their target reproduction depth complicate interpretation of geochemical data from the sediment because some proportion of their shell calcite was added at considerably colder, deeper water depths. Recent B/Ca, U/Ca and $\delta^{11}B$ data from *G. sacculifer* highlight these issues and the problems encountered when analyzing foraminifera that add gam calcite to their shells (Ni et al., 2007).

For paleoceanographers studying the geochemistry of fossil *O. universa* or other species that produce a gametogenic calcite layer, the experimental data presented here is 'good' news. Our new results reduce the amount of gam calcite added to a foraminifera shell from earlier estimates by ~30–50% (in relative terms). Because this calcite layer could be added to the shell surface at a depth that is different from the normal ontogenetic calcite, reconstructions of such species have had to account for a relatively large mass addition (30% or more) at some deeper depth in the water column. Our data now indicate that >80% of *O. universa* shell calcite is ontogenetic. If the amount of gam calcite added to the shells of other species such as *G. sacculifer* is similar to that of *O. universa*, then the interpretation of fossil shell geochemistry for these important species is simplified considerably. However, it should be noted that although the carbon and oxygen stable isotopic composition of ontogenetic and gam calcite are indistinguishable when secreted under identical conditions, that the Mg/Ca ratios are significantly different (Eggins et al., 2004; Nürnberg et al., 1996).

4.1. A possible mechanism for gam calcite addition

In our experiments, the final shell mass of low light *O. universa* was only 60% of the high light specimens, presumably due to the impact of symbiont photosynthesis on shell calcification via elevation of the ambient $[CO_3^{=}]$. In contrast, the contribution of gam calcite to LL *O. universa* was 70% greater than for specimens grown under high light (17% vs. 10%) (Fig. 5, Table 4). A calculation of the amount of gam

calcite added to the shells yields a constant mass addition of 4.2±2.0 μg (n=22) and 4.0±2.4 μg (n=21) for the HL and LL groups respectively (Fig. 6 and Table 4). The relative consistency of gam calcite addition onto shells with different physiological histories suggests gam calcite addition is not a function of the physiological state of the foraminifera prior to spine shortening. Rather, we suggest that these results point to a source of gam calcite that is linked to a volumetrically constant cytoplasmic reservoir that can increase the carbonate saturation state (Ω) of the boundary layer adjacent to the foraminifera shell just before reproduction. In our opinion, there are only two potential cytoplasmic pools that, if released just prior to gametogenesis, could trigger a rapid calcite precipitation event across the entire shell surface. Either the carbonate alkalinity (e.g. $[HCO_3^-]$ or $[CO_3^=]$) or $[Ca^{2+}]$ of the boundary layer against the shell needs to increase to elicit a shift in Ω and the observed addition of gam calcite.

^{14}C tracer investigations have demonstrated the existence of a cytoplasmic carbon pool in a number of perforate, symbiont-bearing benthic foraminifer although imperforate species appear to lack such a carbon concentrating mechanism (ter Kuile and Erez, 1987; ter Kuile et al., 1989a; ter Kuile et al., 1989b; ter Kuile and Erez, 1988). During the *O. universa* experiments reported here, we attempted to identify such a carbon pool in *Orbulina* using ^{13}C-spike pulse-chase experiments (Bijma et al., 1999). Stable isotope analyses of shells maintained in ^{13}C-spiked seawater until the evening before initial sphere calcite precipitation did not record a measurable increase in shell $\delta^{13}C$ values suggesting that a carbon pool does not exist, or if one is present, it must recycle over a short period of time. However, if carbon is concentrated in small vacuoles, vesicles or some other organelle, they are likely to be highly alkaline and a source of $CO_3^=$ if released near the shell surface (Erez, 2003).

The presence/absence of a Ca pool has been investigated previously in *G. sacculifer* and *O. universa*. In a series of ^{45}Ca radiotracer experiments, Anderson and Faber (1984) confirmed the existence of a Ca reservoir in *G. sacculifer* that could account for 1 to 39% ($\bar{x}$=11%) of the shell calcite mass. The size range of shells used in their study (623 to 730 μm) converts to shell masses of roughly 40 to 55 μg (Spero and Lea, 1993). Based on these calculations, the *G. sacculifer* calcium pool could contain sufficient Ca to account for 4.4 to 6.1 μg of calcite if it were released prior to gametogenesis and was entirely incorporated into calcite. Such a mass would be consistent with the gam calcite mass addition estimated for *O. universa*.

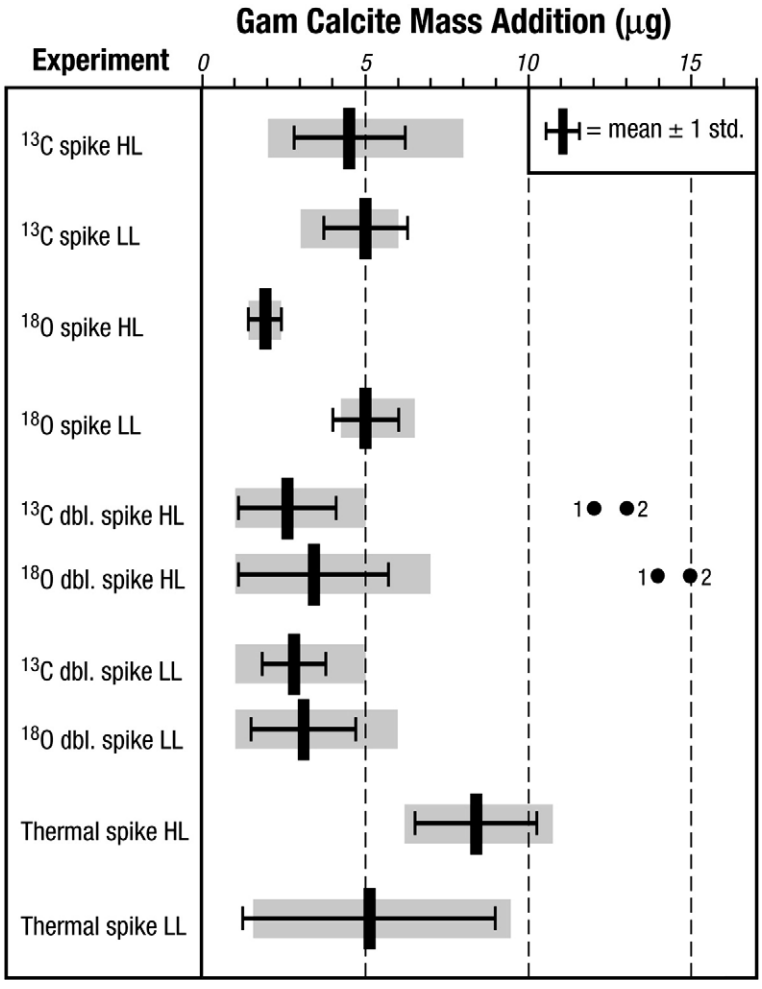

Fig. 6. Mass of gam calcite addition calculated from data in Fig. 5 and individual shell masses.

Lea et al. (1995) investigated the existence of a calcium pool in *O. universa* by conducting pulse-chase experiments with ^{48}Ca labeled seawater. They also found a storage pool, but the [Ca] could only account for 0.15 µg of calcite on average. This estimate was calculated for specimens with newly formed (<19 h) spheres in which the cytoplasmic volume was roughly the volume of the internal juvenile trochospiral shell. If the size of a calcium storage pool is proportional to the volume of cytoplasm inside a sphere, the results of Lea et al. (1995) would reflect the pool size upon initial sphere calcification rather than the pool size at the gam calcite addition stage.

We test this hypothesis by assuming that the cytoplasmic volume of the newly formed spheres in the study of Lea et al. (1995) was the same as the volume of a juvenile trochospiral shell. We also assume that the cytoplasmic volume of a mature *O. universa* with cytoplasm that filled the sphere is equivalent to the volume of a terminal sphere. For these calculations, we use 7×10^6 µm^3 as the volume of a trochospiral shell with a shell length of 349 µm (Spero et al., 1991) and 7 to 8.5×10^7 µm^3 as the mean volume of the spherical chambers that grew in the HL and LL experiments (measured/computed diameters/thicknesses of 544 µm/17 µm and 568 µm/11 µm respectively). The cytoplasmic volume of a pre-gametogenic sphere would therefore be 10–12 times greater than that in a juvenile specimen. Given a proportionality between cytoplasm volume and Ca pool size, we estimate sufficient Ca to account for 1.5–1.8 µg of calcite if the production of gam calcite is entirely attributed to Ca from the Ca pool. This estimate is only ~40% of the calculated gam calcite mass addition in our experiments. Fig. 7 shows the computed shell volume for each individual specimen cultured here, together with the gam calcite addition determined from the spike experiments. It is clear that most experimentally derived gam calcite masses exceed the mean Ca pool-derived estimate of 1.5–1.8 µg. Furthermore, although the largest volume shells had considerably more gam calcite precipitated on the shell surface, a clear volume:gam calcite mass addition relationship is not evident for the remaining data.

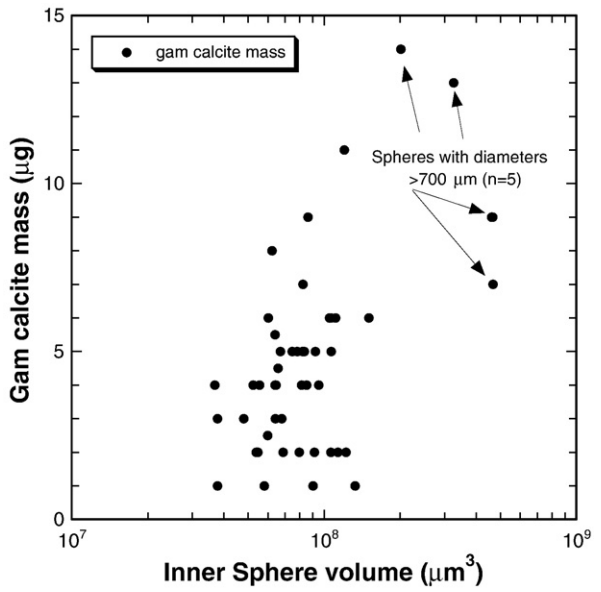

Fig. 7. Sphere volume vs. gam calcite mass addition for individually analyzed *O. universa* shells grown in the spike experiments. Specimens with sphere diameters >700µm are identified with arrows. See text for details.

Although we are unable to directly compute the gam calcite mass in *O. universa* from a cytoplasmic Ca pool volume alone, we recognize the possibility that if both Ca and carbon pools of sufficient size did exist and were released together, they could act synergistically with Ca and alkalinity in the seawater microenvironment adjacent to the shell to produce the additional gam calcite measured on the shell surface. In this regard, we note that a recent microelectrode study conducted on *O. universa* (Kohler-Rink and Kuhl, 2005) recorded Ca concentrations against the shell surface of 10.8 mmol/kg in the light and 10.4 mmol/kg in the dark. Given a seawater [Ca] of 10 mmol/kg and the precision of the Ca microelectrode, their results demonstrate that *O. universa* has the ability to control the Ca concentration in the shell microenvironment, presumably via the release of Ca from an internal pool. From these data we conclude that if a Ca pool of the proper volume exists, we cannot compute its size from sphere volume alone and it appears that cytoplasm volume does not scale proportionately to available sphere volume.

Two questions remain. Where is the calcium pool and if it does play a primary role in gam calcite secretion, then why does a foraminifera expel the pool prior to gametogenesis? The location of the calcium pool in *O. universa* remains enigmatic. An electron micrograph study of *O. universa* biomineralization (Spero, 1988) suggested that calcium storage occurred in a cytoplasm organelle called a fibrillar unit. The evidence was based on TEM observations of fibrillar unit release into the calcifying microenvironment during initial *O. universa* sphere formation. Ultrastructural calcium localization data are not available.

The rationale behind gam calcite production may be related to the cellular changes that take place prior to gamete production. Early studies of gametogenesis in planktonic foraminifera demonstrated that the ultrastructural transition from normal ontogeny to gamete production and subsequent gamete release was preceded by the total reorganization of organelles and parent foraminifera cytoplasm (Bé et al., 1983). Over a period of 12–24 h that follows gam calcite production, a foraminifera produces several hundred thousand flagellated gametes with little remaining evidence of the parent cell organelles in the cytoplasm. It is well known that free calcium is maintained at extremely low levels in cells because it serves a critical role as an intracellular messenger. We hypothesize that the release of the calcium pool evolved to prevent the possible disruption of gamete formation as the foraminifera cytoplasm reorganizes prior to gametogenesis.

If our hypothetical mechanism for gam calcite production is correct, then we are left with the question as to why all planktonic foraminifera do not produce such a layer or calcite crust. Although we do not have a clear explanation, we note that species such as *Globigerina bulloides* or *Globigerinoides ruber*, which do not add a gam calcite layer, tend to produce shells that are much less massive than gam calcite producers even though *G. ruber* is associated with symbiotic dinoflagellates. One possible explanation is that the non-gam calcite species have a reduced calcium or carbon pool in their cytoplasm. Future experiments will be needed to resolve this issue.

5. Conclusions

We have conducted a suite of laboratory experiments with living foraminifera to quantify the contribution of gametogenic calcite to the shell surface of *O. universa*. Results from a combination of ^{13}C, ^{18}O and thermal spike labeling experiments under high and low light conditions demonstrate that the mass of calcite precipitated just prior to gametogenesis makes up between 4 and 20% of the final shell mass. We further demonstrate that the contribution of gam calcite to the shell is significantly greater in foraminifera maintained in a low light environment relative to high light, a function of the higher mass of pre-gam shells precipitated under maximum vs. minimum photosynthetic activity. These results indicate that past studies of gam calcite addition have overestimated the amount of gam calcite on foraminifera shells by ~30–55% (relative value).

Calculation of the calcite mass added to the *O. universa* yields a constant amount of ~4 μg in both the HL and LL experimental groups. We hypothesize that the production of a constant amount of gam calcite may be the result of the discharge of a relatively constant-volume cytoplasmic pool of either Ca^{2+} or alkalinity (carbon pool) that increases the calcite saturation state in the microenvironment adjacent to the foraminifera shell just prior to gamete formation and release. We also suggest that the calcium pool empties just prior to gametogenesis in order to allow gamete formation when the foraminifera cytoplasm reorganizes.

If these results can be extended to other foraminifera species that precipitate gam calcite such as *G. sacculifer* or *G. conglobatus*, then the geochemistry of fossil shells from deep sea sediments is composed of >80% ontogenetic calcite that was precipitated in the primary near surface habitat. As a result, paleoenvironmental interpretations of fossil shell geochemistry from these important species would be simplified considerably.

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