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A universal carbonate ion effect on stable oxygen isotope ratios in unicellular planktonic calcifying organisms

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Abstract

The oxygen isotopic composition ($\delta^{18}O$) of calcium carbonate of planktonic calcifying organisms is a key tool for reconstructing both past seawater temperature and salinity. The calibration of paloeceanographic proxies relies in general on empirical relationships derived from experiments on extant species. Laboratory experiments have more often than not revealed that variables other than the target parameter influence the proxy signal, which makes proxy calibration a challenging task. Understanding these secondary or "vital" effects is crucial for increasing proxy accuracy and possibly for developing new biomarkers. We present data from laboratory experiments showing that oxygen isotope fractionation during calcification in the coccolithophore *Calcidiscus leptoporus* and the calcareous dinoflagellate *Thoracosphaera heimii* is dependent on carbonate chemistry of seawater in addition to its dependence on temperature. A similar result has previously been reported for planktonic foraminifera, suggesting that the $[CO_3^{2-}]$ effect on $\delta^{18}O$ is universal for unicellular calcifying planktonic organisms. The slopes of the $\delta^{18}O/[CO_3^{2-}]$ relationships range between -0.0243 $(\mu mol\,kg^{-1})^{-1}$ (calcareous dinoflagellate *T. heimii*) and the previously published 0.0022 $(\mu mol\,kg^{-1})^{-1}$ (non-symbiotic planktonic foramifera *Orbulina universa*), while *C. leptoporus* has a slope of 0.0048 $(\mu mol\,kg^{-1})^{-1}$. We present a simple conceptual model, based on the contribution of $\delta^{18}O$-enriched HCO_3^- to the CO_3^{2-} pool in the calcifying vesicle, which can explain the $[CO_3^{2-}]$ effect on $\delta^{18}O$ for the different unicellular calcifiers. This approach provides a new insight into biological fractionation in calcifying organisms. The large range in $\delta^{18}O/[CO_3^{2-}]$ slopes should possibly be explored as a means for paleoreconstruction of surface $[CO_3^{2-}]$, particularly through comparison of the response in ecologically similar planktonic organisms.

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1 Introduction

Calcification by marine organisms is a key component of the global carbon cycle. Planktonic calcifying organisms are particularly significant since they are responsible for more than 80 % of global $CaCO_3$ production and a high proportion of their shells is exported to the seafloor to form carbonate sediments (Milliman, 1993).

The unicellular coccolithophore algae, members of the division Haptophyta, are among the major primary producers and calcifiers worldwide. Coccolithophores are present in virtually all photic zone marine environments (Baumann et al., 2005; Thierstein and Young, 2004) and together with planktonic foraminifera they dominate carbonate production in the open ocean (Ziveri et al., 2007; Broecker and Clark, 2008). Another group of organisms that contributes to pelagic carbonate production is a monophyletic lineage of peridiniphycidean dinoflagellates that live in the upper water column where light is available for photosynthesis and during their life cycle produce cysts that are characterized by the incorporation of calcite in at least one layer of the cyst wall.

The chemical composition of the calcite produced by these organisms (i.e. foraminiferal tests, the coccoliths of coccolithophores and the cysts of calcareous dinoflagellates) provides a unique source of information with regard to paleoclimatological studies (e.g. Stoll and Ziveri, 2004). Among other biomarkers associated to calcite, the oxygen isotope composition of carbonate sediments has been used to reconstruct the most sought-after target in paleoceanography, the sea surface temperature (SST). The δ^{18}O-temperature relationship is well characterized in foraminifera (Bemis et al., 1998) and has also been studied in coccolithophores (Ziveri et al., 2003 and references therein) and calcareous dinoflagellate cysts (Zonneveld et al., 2007). In addition to the temperature effect, a dependence of oxygen isotope fractionation on the carbonate chemistry of seawater was demonstrated in four planktonic foraminiferal species (Spero et al., 1997; Bijma et al., 1999). A decrease in fractionation with increasing carbonate ion concentration was recorded in tests of these species, but the slopes for this relationship differed by a factor of up to two. The shallower of the slopes

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was explained in terms of the isotopic composition of the sum of the carbon species in seawater (Zeebe, 1999), but the steeper slope has remained unexplained to date.

The influence of carbonate chemistry on the oxygen isotope fractionation of coccolithophores and calcareous dinoflagellates has never been studied. We therefore conducted controlled laboratory experiments using one of the most important calcite producers among the coccolithophores, *Calcidiscus leptoporus* (Ziveri et al., 2007), as well as the most abundant living calcareous dinoflagellate, *Thoracosphaera heimii*. The carbonate chemistry of seawater was altered to monitor its impact on oxygen isotope fractionation during calcification. In addition to our experimental data, we propose a simple conceptual model that can explain the different $\delta^{18}O$ slopes in terms of a different contribution of HCO_3^- conversion to CO_3^{2-} to the establishment of supersaturation with respect to calcite in the calcification vesicle. The applicability of this model to data previously obtained from studies on planktonic foraminifera (Spero et al., 1997; Bijma et al., 1999) is discussed.

2 Culture experiments

2.1 Experimental design and sampling

Clonal cultures of *Calcidiscus leptoporus* (strains RCC1154, formerly known as AC360 and AS31 from the Alboran Sea off Spain, and RCC1135 formerly known as AC365 and NS6-1 from the South Atlantic off South Africa) and of *T. heimii* (strain RCC1511 formerly known as JF1 from the Pacific Ocean) from the Roscoff Culture Collection, (http://www.sb-roscoff.fr/Phyto/RCC/) were grown in sterile filtered (0.2 µm) seawater enriched with 100 µM nitrate and 6.25 µM phosphate and with trace metal and vitamin supplements according to f/2(-Si) (Guillard and Ryther, 1962). The incident photon flux density was 350 µmol m^{-2} s^{-1} and a 16/8 h light/dark cycle was applied. Experiments were carried out at 20 °C for *C. leptoporus* and 17 °C for *T. heimii*. Cells were acclimated to experimental conditions for approximately 10 generations and grown in dilute batch

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cultures in triplicate (Langer et al., 2006). Low cell density at harvest (in general less than 6000 cells per ml) resulted in less than 8 % DIC (dissolved inorganic carbon) consumption (i.e. DIC consumed by the cells at the end of experiment) and a shift in pH of no more than 0.06 units.

The carbonate system of seawater can be manipulated in various ways. Changes in atmospheric pCO_2 can be used to alter [CO_2], pH and DIC, with TA (total alkalinity) remaining constant. Manipulating pCO_2 requires bubbling with CO_2 gas (constantly throughout the experiment if an open system is employed). Alternatively, the addition of the acid HCl or the base NaOH can be used to adjust [CO_2], pH, and TA, with DIC remaining constant. Both methods, TA manipulation (*C. leptoporus*) and DIC manipulation (*T. heimii*), were employed in this study, in both cases in closed systems (see below). It should be noted that chemical changes imposed by the two methods are of similar magnitude. The pCO_2 range typically used in experiments is ~180 to ~1000 µatm. This range can be covered by either increasing DIC by approximately 15 % or decreasing TA by approximately 15 % (to increase CO_2). The change in either DIC or TA is small compared to the changes in CO_2 concentration at the upper limit (increase by a factor of ~6), CO_3^{2-} concentration (decrease by a factor of ~4), and H^+ concentration (increase by a factor of ~5, i.e. a pH drop of ~0.7 units) (for details on the carbonate system refer to Zeebe and Wolf-Gladrow, 2001). Experiments in which DIC is altered should thus be comparable to experiments in which TA is altered (Schulz et al., 2009; Hoppe et al., 2011).

In order to prevent gas exchange with the atmosphere in our experiments, 2.4 L flasks were filled without headspace and closed with Teflon-lined screw caps. Determination of cell density, however, required regular sampling for cell counts, thereby creating a maximum headspace of 6 ml, and resulting in a negligible shift (3 %) in CO_2(aq) concentration. Samples for alkalinity measurements were filtered (0.6 µm pore size), poisoned with 1 ml of a $HgCl_2$ solution (35 g l^{-1}) and stored in 300 ml borosilicate flasks at 0 °C. DIC samples were sterile filtered using a syringe filter (0.2 µm) and stored in 13 mL borosilicate flasks free of air bubbles at 0 °C. Total alkalinity was calculated

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from linear Gran plots (Gran, 1952) after duplicate potentiometric titration (Bradshaw et al., 1981; Brewer et al., 1986) and DIC was measured photometrically (Stoll et al., 2001) in triplicate. Precision of the total alkalinity measurements was ~3 µmol l^{-1} and accuracy ~4 µmol l^{-1}. For dissolved inorganic carbon, precision was ~4 µmol l^{-1} and accuracy ~5 µmol l^{-1}. The carbonate system was calculated from temperature, salinity, and the concentrations of DIC, total alkalinity and phosphate, using the CO_2 sys software (Lewis and Wallace, 1998). Equilibrium constants of Mehrbach et al., 1973 refitted by Dickson and Millero, 1987 were chosen.

Cell samples for oxygen and carbon isotope analysis were centrifuged in 50 ml Falcon tubes, centrifuged again in 1 ml tubes in order to remove seawater, dried at 60 °C for 48 h, and stored at room temperature. After the last centrifugation step, care was taken to remove as much of the seawater medium as possible in order to minimize contamination due to residual salts. Seawater samples for isotope analysis were sterile filtered (0.2 µm), poisoned with HgCl, and stored in gas tight bottles at room temperature prior to analysis. For determination of cell density, samples were taken daily or every other day, stored at 0 °C and counted within 3 h of sampling using a Sedgwick Rafter counting cell.

2.2 Sample preparation and measurement of isotope ratios

The $\delta^{18}O$ of water samples from the *C. leptoporus* experiments was measured at the Leibniz Laboratory, Kiel University, on a Finnigan Delta E connected with an equilibration bath (Equi). An aliquot of the sample water was isotopically equilibrated with an external CO_2, this CO_2 was extracted, analyzed by mass spectrometer, and $\delta^{18}O$ calculated from the isotope composition. $\delta^{18}O$ calibration of the working reference gas was based on VSMOW, with a fine correction derived from analysis of VSMOW, GISP, and SLAP. This calibration was performed once or twice a year. The stability control in routine operation used internal water standards of different isotopic composition. As with the DIC sample series, a second gas standard was measured at the end of each sample batch. The 1σ standard deviation of water samples was ± 0.05 for

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δ^{18}O. The poisoned filtered medium samples from the *T. heimii* culture experiments were measured at the Vrije Universiteit (Amsterdam) using an on-line gas preparation (Finnigan GasBench II). The 1σ standard deviation of water samples for δ^{18}O was ± 0.05. Reproducibility tests were based on a routinely run laboratory water standard (Vienna-Standard Mean Ocean Water (V-SMOW)). δ^{18}O values ranged from −0.33 to 0.09 ‰ (SMOW).

The stable isotopes of coccolith and calcareous dinoflagellate samples were measured at the Vrije Universiteit (Amsterdam) using a CARBO-KIEL automated carbonate preparation device linked on-line to a Finnigan MAT252 mass spectrometer, equipped with an automated carbonate extraction line (Kiel device). The reproducibility of a routinely analyzed carbonate standard (GICS) was better than 0.15 ‰ for δ^{18}O for the MAT252. Based on replicate analyses of splits of culture samples, the mean reproducibility of the δ^{18}O measurements was better than ± 0.2 ‰.

3 Experimental results and discussion

Carbonate ion concentration in the seawater medium in which the cells were grown, calculated using CO_2sys (Lewis and Wallace, 1998), ranged from 78 to 530 µmol kg^{-1} for *C. leptoporus* experiments and from 117 to 239 µmol kg^{-1} for *T. heimii* experiments. The δ^{18}O in coccoliths of *C. leptoporus* was inversely correlated with the computed CO_3^{2-} concentration ($r^2 = 0.89$, with a regression slope of −0.0048 ± 0.02 ‰ (µmol kg^{-1})$^{-1}$) (Fig. 1). The δ^{18}O in *T. heimii* calcite exhibited the same type of inverse relationship with CO_3^{2-} concentration, but with a regression slope of −0.0243 ± 1.74 ‰ (µmol kg^{-1})$^{-1}$ ($r^2 = 0.95$).

The slopes of the δ^{18}O/[CO_3^{2-}] relationships for tested planktonic calcifiers range from 0.0022 (µmol kg^{-1})$^{-1}$ for the foraminifer *Orbulina universa* to 0.0243 (µmol kg^{-1})$^{-1}$ for the calcareous dinoflagellate *T. heimii* (Fig. 1). *T. heimii* has an anomalously steep slope which suggests strong biological control on oxygen isotope fractionation. Of

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the species analyzed to date, *T. heimii* shows the largest dissimilarity in $\delta^{18}O/[CO_3^{2-}]$ slope between biological and inorganic precipitates (Spero et al., 1997; Zeebe, 1999). The heavily calcified coccolithophore *C. leptoporus* has a $\delta^{18}O/[CO_3^{2-}]$ slope of 0.0048 $(\mu mol\,kg^{-1})^{-1}$, similar to that for inorganic precipitates and for the planktonic foraminifer *G. bulloides*, but steeper than the slope reported for *O. universa* (Spero et al., 1997).

In coccolithophores, precipitation of calcite takes place intracellularly within a calcifying vesicle, a membrane-delimited space that is completely isolated from the cytoplasm (e.g. Young et al. 1999). While the pH in the external medium can change under different environmental conditions, cellular pH-homeostasis should keep the pH inside the vesicle constant at an alkaline value favorable for calcite precipitation. Zuddas and Mucci (1998) and Kim et al. (2006) demonstrated that for an alkaline pH, the kinetics of $CaCO_3$ precipitation are dominated by the reaction: $Ca^{2+} + CO_3^{2-} = CaCO_3$. Assuming that calcium carbonate is precipitated mainly from carbonate, the $\delta^{18}O$ composition of the precipitate simply reflects the isotopic composition of CO_3^{2-} in the vesicle. We assume that the uptake of both CO_3^{2-} and HCO_3^- from the surrounding medium contribute to the establishment of the CO_3^{2-} pool in the vesicle. The uptake of CO_3^{2-} is assumed to yield a pool size proportional to $[CO_3^{2-}]$ in the external medium (ext): $f \times [CO_3^{2-}]_{ext}$ with $f \leq 1$. Inorganic carbon transported into the vesicle (V) as HCO_3^- is converted to CO_3^{2-}, and the $[CO_3^{2-}]_V$ increases until supersaturation with respect to calcite is obtained in the vesicle and calcium carbonate starts to precipitate at $[CO_3^{2-}]_V = [CO_3^{2-}]_{sat}$. The contribution of bicarbonate conversion to the CO_3^{2-} pool in the vesicle is given by: $[CO_3^{2-}]_{sat} - f \times [CO_3^{2-}]_{ext}$ (Fig. 2). The H^+ generated during CO_3^{2-} formation from HCO_3^- has to be removed from the vesicle to keep the pH value of the calcifying vesicle constant.

Chemical equilibrium in the intracellular carbon dioxide system is obtained after several seconds (approximately 15 s at seawater pH = 8.2 and 25 °C, Zeebe et al., 1999), which is negligible compared with the 1.4 h (calculated using data in Langer et al., 2006) required for the formation of one coccolith (of *C. leptoporus*). The time to

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establish $\delta^{18}O$ equilibrium, on the other hand, is one order of magnitude higher than the time required for coccolith formation (see table 3.3.9 of Zeebe and Wolf-Gladrow, 2001). It is therefore reasonable to assume that the carbon dioxide system in the calcifying vesicle approaches its chemical equilibrium, whereas the CO_3^{2-} in the vesicle will carry the isotopic fingerprint of the inorganic carbon transported from the cytoplasm into the vesicle. Then, the oxygen isotope fractionation factor between the CO_3^{2-} in the vesicle and water is given by:

$$\alpha_{(V-H_2O)} = \left(\alpha_{\left(CO_3^{2-}-H_2O\right)} \times f \times \left[CO_3^{2-}\right]_{ext} + \alpha_{(HCO_3^{-}-H_2O)} \times \left(\left[CO_3^{2-}\right]_{sat} - f \times \left[CO_3^{2-}\right]_{ext}\right)\right) \Big/ \left[CO_3^{2-}\right]_{sat} \quad (1)$$

Two terms contribute to Eq. (1): the first term represents the equilibrium isotopic composition of CO_3^{2-}, whereas the second term is the isotopic composition of the ^{18}O-enriched HCO_3^- flux into the calcifying vesicle. Here, we propose an explanation of the different $\delta^{18}O/[CO_3^{2-}]$ slopes for unicellular calcifying organisms in terms of different availability of external carbonate ($f \times [CO_3^{2-}]_{ext}$) for the establishment of supersaturation with respect to calcite in the vesicle ($[CO_3^{2-}]_V = [CO_3^{2-}]_{sat}$). The $\delta^{18}O$ of CO_3^{2-} in the vesicle decreases with increasing external carbonate ion concentration since the contribution of bicarbonate conversion ($[CO_3^{2-}]_{sat} - f \times [CO_3^{2-}]_{ext}$) to the CO_3^{2-} pool in the vesicle decreases as $[CO_3^{2-}]_{ext}$ rises. According to this process, the $\delta^{18}O$ slope is influenced by the calcite saturation product (K_{sp}) in the calcifying vesicle: $K_{sp} = [CO_3^{2-}]_{sat} \times [Ca^{2+}]_{sat}$. The $\delta^{18}O/[CO_3^{2-}]$ slope becomes steeper with decreasing K_{sp}, which is a function of temperature and salinity (Mucci, 1983).

It is still a matter of debate which compartment of the cell takes up Ca^{2+} from the cytoplasm. It has been suggested that the peripheral endoplasmic reticulum (ER) could play this role (Berry et al., 2002). Using the seawater saturation product ($K_{sp} = 10^{-6.415}\,mol^{-2}\,kg^{-2}$ at $S = 32$ and $T = 20\,^{\circ}C$) and for $[Ca^{2+}]_{sat}$ a typical value for $[Ca^{2+}]$ in the ER ($500\,\mu mol\,kg^{-1}$; Meldolesi and Pozzan, 1998) yields a $[CO_3^{2-}]_{sat}$ in the vesicle of $769\,\mu mol\,kg^{-1}$. The oxygen isotope fractionation factors (α)

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between dissolved inorganic carbon species and water can be calculated using the equations derived by Beck et al. (2005). In freshwater at 20 °C (applicable to *C. leptoporus* experiments), the fractionation factors between HCO_3^-, CO_3^{2-} and water are $\alpha_{(HCO_3^- - H_2O)} = 1.0325$ and $\alpha_{(CO_3^{2-} - H_2O)} = 1.0254$. At 17 °C (*T. heimii* experiments), the freshwater fractionation factors are $\alpha_{(HCO_3^- - H_2O)} = 1.0332$ and $\alpha_{(CO_3^{2-} - H_2O)} = 1.0260$. We applied Eq. (1) using the freshwater α factors at 20 °C to simulate the $\delta^{18}O/[CO_3^{2-}]$ slope for *C. leptoporus*. The measured slope of coccolith $\delta^{18}O$ vs. $[CO_3^{2-}]$ coincides with the calculated slope for CO_3^{2-} in the vesicle (-0.0048 $(\mu mol\,kg^{-1})^{-1}$, see Fig. 1) assuming $f = 0.53$ (for definition of f see above). The model also applies to other calcifiers that possess a vesicle-based calcification mechanism, such as the foraminifera *O. universa* and *G. bulloides*. The shallower slope of *O. universa* (-0.0022 $(\mu mol\,kg^{-1})^{-1}$) is obtained by specifying an f factor of 0.24, a result that indicates that a considerable amount of CO_3^{2-} in the vesicles of *O. universa* is derived from bicarbonate conversion.

Note that while $\delta^{18}O$ slopes can be calculated using Eq. (1), absolute $\delta^{18}O$ values of CO_3^{2-} in the vesicle, and thereby of the precipitated calcite, cannot be compared. The reason for this is that fractionation factors provided by Beck et al. (2005) refer to freshwater, whereas cells were grown at a salinity of 32. However, the seawater saturation product (at $S = 32$) is not necessarily appropriate for understanding calcite precipitation and ^{18}O fractionation in unicellular calcifying marine organisms. Coccolithophores, for instance, exert strong control over the chemical composition of the calcifying fluid. The ionic strength inside the coccolith vesicle is most likely lower than that of seawater (Anning et al., 1996). In fact, the very steep $\delta^{18}O$ slope observed in culture experiments with *T. heimii* (-0.024 $(\mu mol\,kg^{-1})^{-1}$) could be explained in terms of a salinity at the site of calcification (S_V) of 14.64, which is less than half of the salinity in seawater. Based on the saturation product ($K_{sp} = 10^{-6.803}\,mol^{-2}\,kg^{-2}$ at $S_V = 14.64$ and $T = 17$ °C), a $[Ca^{2+}]_{sat}$ of 500 $\mu mol\,kg^{-1}$ would correspond to a $[CO_3^{2-}]_{sat}$ in the vesicle of 315 $\mu mol\,kg^{-1}$. Using this value for $[CO_3^{2-}]_{sat}$ in the vesicle and the freshwater

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α factors at 17°C, the slope of calcite $\delta^{18}O$ vs. $[CO_3^{2-}]$ can be fitted using Eq. (1) by assuming a maximum access to the external CO_3^{2-} during calcite precipitation in the vesicle, i.e. $f = 1$ (see Fig. 2).

In conclusion, the results presented here show that the negative slope of the $\delta^{18}O$ /$[CO_3^{2-}]$ relationship that has been recorded in planktonic foraminifera is also exhibited by two phylogenetically remote groups of unicellular calcifiers, namely coccolithophores and dinoflagellates. The negative slopes are explained for the first time by a conceptual model that takes physiological mechanisms into consideration. According to the model, the dependence of $\delta^{18}O$ on carbonate chemistry is mediated through a contribution of HCO_3^- to the CO_3^{2-} pool in the calcifying compartment.

Previous attempts have been made to correct the foraminiferal $\delta^{18}O$ proxy for variations of paleo-$[CO_3^{2-}]$ using the experimental slopes during time-windows when seawater carbonate chemistry was notably different. The new results demonstrate similar $\delta^{18}O/[CO_3^{2-}]$ slopes (0.0048 to 0.0022 $(\mu mol\,kg^{-1})^{-1}$) for planktonic foraminifera and coccolithophores. A strong "vital effect" is indicated by the steep slope recorded for a calcareous dinoflagellate. There is considerable scope for further exploration of oxygen isotope composition as a tool for paleo-$[CO_3^{2-}]$ reconstruction by comparison of coccolithophore and dinoflagellate taxa that share (or shared) the same habitat. When contribution of calcareous dinoflagellates to the carbonate fraction is pronounced, the $\delta^{18}O/[CO_3^{2-}]$ relationship in this group could also contribute to the understanding of bulk $\delta^{18}O$ variability during periods of rapidly changing seawater carbonate chemistry. With the proposed model in mind, further comparative studies on other species of these groups of planktonic calcifiers could provide important mechanistic insights into intra- and inter-group similarities and/or differences in calcification processes. Such studies would also help to assess the degree of bias introduced into $\delta^{18}O$-based SST reconstructions by past changes in ocean carbonate chemistry.

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Table 1. Experimental data from this study: CO_2 (µatm), $CO_3^=$ (µmol kg^{-1}), Omega calcite saturation, pH (total scale) values, α and ε fractionation factors (calcite-water) and δ^{18}Oc-δ^{18}Ow (calcite – water) (PDB).

species	CO_2 µatm	$CO_3^=$ µmol Kg	Omega calcite	pH total scale	$\alpha(x\text{-}H_20)$	$\varepsilon(x\text{-}H_20)$	δ^{18}Oc- $\delta^{18}OH_20$ (PDB)
Calcidiscus leptoporus	953.3	78.5	1.95	7.84	1.0278	27.84	−2.66
Calcidiscus leptoporus	914.6	82.6	2.06	7.86	1.0278	27.76	−2.73
Calcidiscus leptoporus	891.6	84.3	2.1	7.87	1.0278	27.79	−2.71
Calcidiscus leptoporus	636.00	103.4	2.51	8	1.0283	28.29	−2.22
Calcidiscus leptoporus	617.00	105.4	2.56	8	1.0283	28.26	−2.25
Calcidiscus leptoporus	619.00	105.6	2.56	8	1.0284	28.35	−2.16
Calcidiscus leptoporus	377.00	161.7	3.92	8.24	1.0277	27.68	−2.81
Calcidiscus leptoporus	379.00	162.8	3.95	8.24	1.0279	27.87	−2.63
Calcidiscus leptoporus	369.00	166	4.03	8.24	1.0278	27.79	−2.71
Calcidiscus leptoporus	350.3	199.7	4.97	8.26	1.0275	27.45	−3.03
Calcidiscus leptoporus	351.1	200.1	4.98	8.26	1.0276	27.61	−2.89
Calcidiscus leptoporus	332.6	207.9	5.17	8.28	1.0277	27.71	−2.79
Calcidiscus leptoporus	262.00	214.1	5.2	8.4	1.0274	27.38	−3.11
Calcidiscus leptoporus	256.00	219.2	5.32	8.4	1.0274	27.39	−3.10
Calcidiscus leptoporus	237.00	233.1	5.66	8.4	1.0274	27.42	−3.07
Calcidiscus leptoporus	104.2	493.8	12.29	8.72	1.0258	25.78	−4.66
Calcidiscus leptoporus	93	530.2	13.19	8.76	1.0261	26.11	−4.34
Thoracosphaera heimii	205.3	239.4	3.74	8.28	1.0273	27.29	−3.19
Thoracosphaera heimii	208	238.2	3.72	8.01	1.0272	27.19	−3.29
Thoracosphaera heimii	439.9	146.4	2.29	8.01	1.0297	29.46	−0.52
Thoracosphaera heimii	464.1	140	2.19	7.99	1.0296	30.04	−1.09
Thoracosphaera heimii	599.4	117.7	1.84	7.9	1.0303	30.33	−0.24
Thoracosphaera heimii	603.2	116.3	1.82	7.89	1.0298	29.82	−0.74
Thoracosphaera heimii	653.8	110.2	1.72	7.86	1.0301	30.10	−0.47

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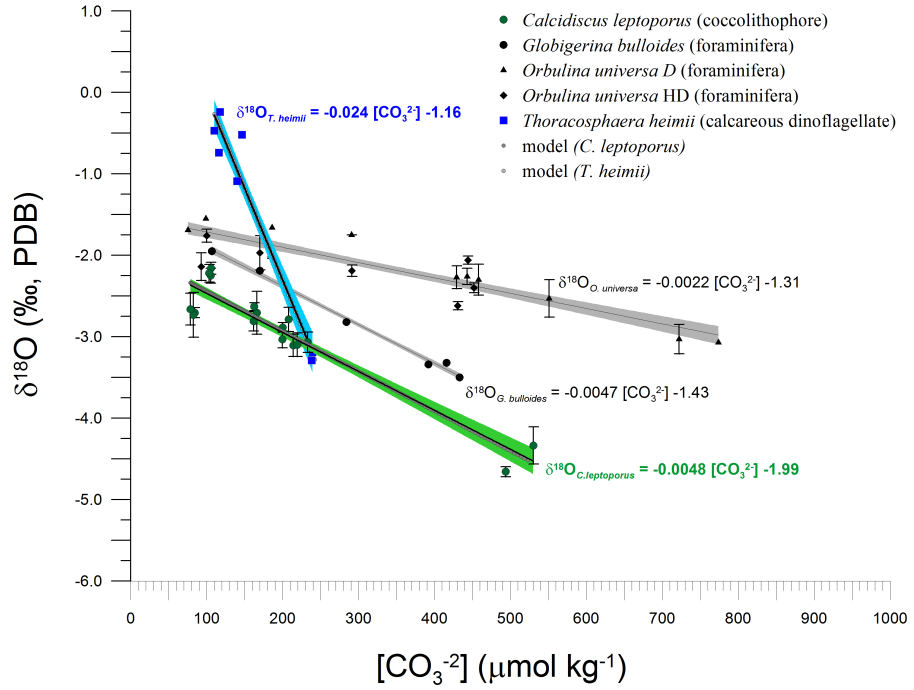

Fig. 1. Effect of $[CO_3^{2-}]$ on $\delta^{18}O$ (δ^{18}Ocalcite-δ^{18}Owater) values of *C. leptoporus* (coccolithophore), *T. heimii* (calcareous dinoflagellate), *O. universa* (HL = foraminifera grown under photosynthetic maximum light levels, D = maintained in the dark) (Spero et al., 1997), and *G. bulloides* (Spero et al., 1997) (lines are linear regressions fitted to the data). The regression slopes are −0.0022 ‰ $(\mu mol\,kg^{-1})^{-1}$ for *O. universa* (HL = high light; DC = dark conditions), −0.0048 ‰ $(\mu mol\,kg^{-1})^{-1}$ for *C. leptoporus*, −0.0047 ‰ $(\mu mol\,kg^{-1})^{-1}$ for *G. bulloides,* and −0.024 ‰ $(\mu mol\,kg^{-1})^{-1}$ for *T. heimii.* 1σ confidence bounds are shown for each regression (shaded areas).

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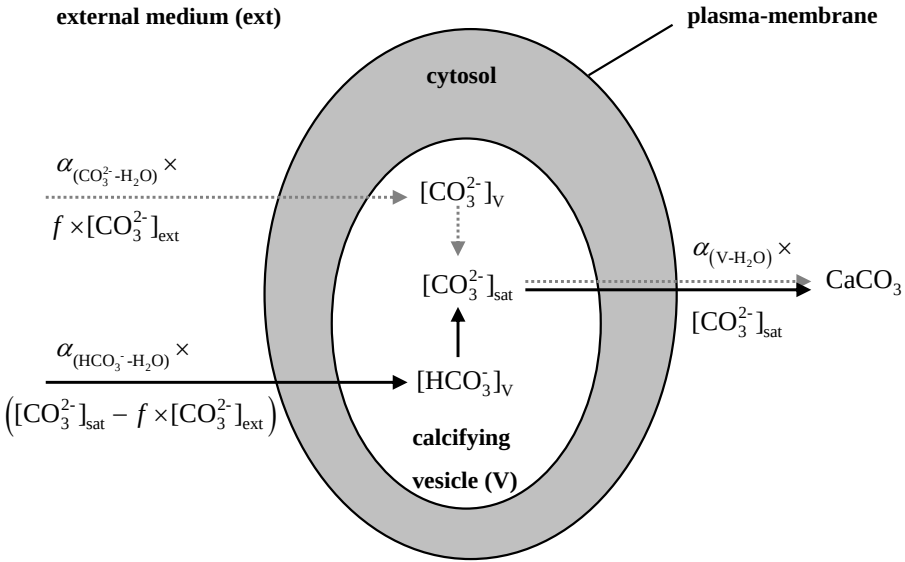

Fig. 2. Proposed fractionation mechanism. Calcium carbonate is precipitated mainly from carbonate, therefore the $\delta^{18}O$ composition of the precipitate simply reflects the isotopic composition of CO_3^{2-} in the vesicle. The uptake of both CO_3^{2-} and HCO_3^- from the surrounding medium contributes to the establishment of the CO_3^{2-} pool in the vesicle. The uptake of CO_3^{2-} is assumed to yield a pool size proportional to $[CO_3^{2-}]$ in the external medium (ext): $f \times [CO_3^{2-}]_{ext}$ with $f \leq 1$. The inorganic carbon transported into the vesicle (V) as HCO_3^- is converted to CO_3^{2-}, and the $[CO_3^{2-}]_V$ increases until supersaturation with respect to calcite is obtained in the vesicle and calcium carbonate starts to precipitate at $[CO_3^{2-}]_V = [CO_3^{2-}]_{sat}$. The contribution of bicarbonate conversion to the CO_3^{2-} pool in the vesicle is given by: $[CO_3^{2-}]_{sat} - f \times [CO_3^{2-}]_{ext}$.

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