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Isotopic partitioning between scallop shell calcite and seawater: Effect of shell growth rate

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Abstract—The relationship between molluscan shell growth rate and skeletal $\delta^{18}O$ and $\delta^{13}C$ was investigated in a detailed field study for the scallop, *Pecten maximus*. Seasonal variation in shell growth rate was found to be a governing factor influencing shell $\delta^{18}O$ and $\delta^{13}C$. At low shell growth rates, shell $\delta^{18}O$ were more positive (of the order +0.4‰) and $\delta^{13}C$ more negative (up to −2‰) as compared with predicted values for precipitation of inorganic calcite in isotopic equilibrium with seawater. The deviations in $\delta^{18}O$ were hypothesized as reflecting possible differences in solution carbonate chemistry at the site of mineralization in the extrapallial fluid as compared with that of the external seawater medium. The deviations in shell $\delta^{13}C$ were consistent with incorporation of isotopically depleted respiratory ^{13}C (i.e., a metabolic effect). A trend toward more depleted shell $\delta^{18}O$ and $\delta^{13}C$ values occurred at higher shell growth rates, with negative $\delta^{18}O$ values as compared with predicted equilibrium at shell growth rates above 0.13 mm per day. These simultaneous negative deviations in skeletal $\delta^{18}O$ and $\delta^{13}C$ were interpreted as resulting from a kinetic effect. The implications for environmental reconstruction from molluscan isotopic records are discussed in light of a model of isotopic behavior based on the findings of the study.

1. INTRODUCTION

The potential for establishing high-resolution records of environmental change from naturally occurring stable isotopes in molluscan shell carbonates is well recognized (Epstein et al., 1953; Weidman et al., 1994; Johnson et al., 2000). Profiles of calcite stable oxygen isotopic composition ($\delta^{18}O$) taken along the axis of shell growth may potentially be used to establish high-resolution records of water temperature and the oxygen isotopic composition of seawater ($\delta^{18}O$-H_2O) (Urey, 1947; McCrea, 1950; Epstein et al., 1953; O'Neil et al., 1969), the latter varying with salinity. Similarly, profiles of calcite stable carbon isotopic composition ($\delta^{13}C$) may potentially be used to establish records of the carbon isotopic composition of dissolved inorganic carbon ($\delta^{13}C$-ΣCO_2) (Romanek et al., 1992) and thereby proxy records of the concentration of dissolved inorganic carbon, [ΣCO_2], and variations in marine productivity. The scallop, an almost entirely calcitic epifaunal bivalve, is an excellent case organism for study because it is well represented throughout the geologic record at all latitudes (Barrera et al., 1990; Smith, 1991; Bice et al., 1996). Indeed, several studies have aimed to establish such proxy records from both contemporary and fossil scallop shell isotopic data (Krantz et al., 1984, 1987; Tan et al., 1988; Dare and Deith, 1989; Johnson et al., 2000).

Partitioning of oxygen isotopes between molluscan shells and seawater has historically been assumed to closely approximate equilibrium isotopic partitioning between inorganic calcite or aragonite and water. For calcitic molluscs, this was originally confirmed by the close agreement of the Epstein et al. (1953) empirically derived expression with that for inorganic calcite (McCrea, 1950; O'Neil et al., 1969). More recently, Kim and O'Neil (1997) comprehensively redetermined oxygen isotope fractionation between inorganic calcite and water, revealing substantial differences with the earlier inorganic calcite expressions over the oceanic temperature range (10 to 25°C). Shell $\delta^{18}O$ for the scallop *Pecten maximus* cultured under laboratory conditions by Owen et al. (2002) exhibited enrichments of the order of +0.6‰ with respect to predicted equilibrium as determined from Kim and O'Neil (1997). This was hypothesized as reflecting possible differences in solution carbonate chemistry at the site of mineralization within the extrapallial fluid (EPF), as compared with the external seawater. Observed depletions in shell $\delta^{13}C$ (of the order of −2.0‰) with respect to predicted values for calcite precipitated in isotopic equilibrium with inorganic calcite (Romanek et al., 1992; Zhang et al., 1995) were interpreted as reflecting introduction of ^{13}C-depleted respiratory CO_2 into the EPF (i.e., a metabolic effect).

In the present study, we have further investigated partitioning of isotopes between scallop shell calcite and seawater in a constrained, year-long field study. The range of shell growth rates exhibited by the scallops in the field allowed us to evaluate further the experimental data, which was associated with low growth rates over the natural temperature range of the organism. It also allowed a detailed investigation of the potential variation in metabolic effect and kinetic effects that have been suggested to occur at higher accretion rates (McConnaughey, 1989; McConnaughey et al., 1997). The current study provides evidence that the initial observations of enrichments of shell $\delta^{18}O$ and depletions of shell $\delta^{13}C$ with respect to equilibrium at low shell growth rates are reproducible. It also shows that shell growth rate is a major factor influencing $\delta^{18}O$ and $\delta^{13}C$. We conclude with a model describing the shell isotopic data and discuss the implications for environmental reconstruction from molluscan isotopic records.

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2. MATERIALS AND METHODS

2.1. Growth of Scallops in the Field for Isotopic Analyses

During the year October 1994 to September 1995, juvenile *Pecten maximus* (<1 yr of age, 1.8- to 2.1-cm shell height and from one spat cohort) were placed in specially designed boxes in the sea (the Menai Strait between the island of Anglesey and the U.K. mainland). Each box, similar in design to that used by Richardson et al. (1980), was suspended 1 m below a moored raft, and a Tinytalk seawater temperature logger (Orion Components, Chichester, U.K.) was mounted in the box before placement in the field. Shell heights (umbo to valve margin) of individual scallops were measured with a binocular microscope fitted with an eyepiece graticule, and each shell was identified by means of a numbered tag glued to the upper flat shell valve. Scallops were then glued by their convex valves to one of three vertical Perspex plates that fitted into the center of the raft box. The Perspex unit was placed in running seawater overnight to allow the scallops to recover before transfer to the raft the next day. Richardson (1990) has shown that a clearly visible growth check is formed in the shells of bivalves exposed to this type of handling to which all subsequent growth can be related. Eight deployments of scallops were made between September 1994 and October 1995, each of approximately monthly duration (hereafter termed "monthly scallops"). For comparison, a concurrent deployment of scallops was made in September 1994, which was left out continuously for a period of 12 months (hereafter termed "annual scallops").

At the end of each deployment, the boxes were retrieved, and the scallops were removed from the Perspex plates and immediately frozen. Before analysis, all fouling organisms were carefully removed from the shells. Macroscopic examination of the shells revealed that nearly all the scallops retrieved after the assigned period in the field displayed a clear disturbance-related growth check on the upper shell valve corresponding to the initial shell height measurements. Scallops that did not display a clear growth check were excluded from the analyses.

For monthly scallops, the increment of shell deposited at the midline was measured on the upper valve for each individual with a binocular microscope with an eyepiece graticule, enabling calculation of shell-growth rate (mm d^{-1}). Calcite samples (>1 mg) were obtained with a dentist's drill fitted with a 0.5-mm dental burr. Samples were obtained from monthly scallops either by drilling the entire growth increment if growth was slow, a subsection of the increment at the midline between disturbance mark and valve margin if growth was intermediate, or by drilling sequential samples at 1.5-mm intervals from the growth check to the valve margin at the midline (isotopic profiling) when growth was fast. Two of the annual scallops were selected for isotopic profiling in the same manner as the fast-growing monthly scallops above. All calcite samples were pretreated to remove organic components in a low-temperature oxygen plasma (Swart, 1981) and subsequently analyzed by sealed vessel acidification method (McCrea, 1950) with a VG SIRA2 dual-inlet isotope ratio mass spectrometer, calibrated against VPDB with NBS-19. Analytical variability associated with $\delta^{18}O$ and $\delta^{13}C$ data for carbonate samples was $1\sigma = 0.08‰$ and 0.04‰, respectively.

2.2. Analyses of Menai Strait Seawater

Measurements of biogeochemical parameters were made at Menai Bridge Pier (located very close to the moored raft) every 1 to 2 weeks between September 1994 and October 1995. Temperature, salinity, $\delta^{18}O\text{-}H_2O$, $\delta^{13}C\text{-}\Sigma CO_2$, [$\Sigma CO_2$], pH, and the concentrations of dissolved inorganic carbon species were determined following the methods outlined by Owen (1998). Concentrations of chlorophyll and dissolved nutrients were determined by the methods outlined by Parsons et al. (1984).

Seasonal predicted $\delta^{18}O_{(equilib)}$ data (the Craig-corrected ^{18}O:^{16}O of calcite precipitated in isotopic equilibrium with seawater on the PDB scale, corrected for the isotopic composition of seawater) were compared with *Pecten* $\delta^{18}O$ data. To calculate $\delta^{18}O_{(equilib)}$, calcite oxygen isotopic data reported against SMOW in Kim and O'Neil (1997) were first converted to the PDB scale (Friedman and O'Neil, 1977) after correcting for differences in the acid fractionation factors used in our study (1.01025) and that of Kim and O'Neil (1997) (1.01050). $\delta^{18}O\text{-}H_2O$ data collected in this study and that of Kim and O'Neil (1997) (both reported on the SMOW scale) were similarly converted to the PDB scale (δ_w, the Craig-corrected ^{18}O:^{16}O of CO_2 equilibrated with the water relative to that of CO_2 from PDB) (Friedman and O'Neil, 1977), a correction of −0.22‰, thereby allowing comparison with earlier studies. A linear regression of the converted $\delta^{18}O\text{-}\delta_w$ against temperature data presented by Kim and O'Neil (1997) was used to calculate predicted $\delta^{18}O_{(equilib)}$ data by use of seasonal environmental δ_w and water temperature data. Bemis et al. (1998) show the appropriateness of the use of linear vs. quadratic temperature/$\delta^{18}O$ relationships at warm oceanic temperatures (9 to 24°C). Recent studies (Spero et al., 1997; Bemis et al., 1998; Zeebe, 1999) have shown the impact of aqueous carbonate ion concentrations on $\delta^{18}O$ data. Applying a correction factor of −0.002‰ per μmol kg^{-1} $CO_{3\,(aq)}^{2-}$ to the $\delta^{18}O_{(equilib)}$ data (Bemis et al., 1998) to account for seasonal variations in [$CO_{3\,(aq)}^{2-}$] resulted in only negligible corrections (mean, 0.028‰ for the entire data set; $1\sigma = 0.05‰$).

Seasonal predicted $\delta^{13}C$ data for calcite precipitated in isotopic equilibrium with seawater ($\delta^{13}C_{(equilib)}$) were established first by determining the seasonal isotopic compositions of $CO_{2(aq)}$, $HCO_{3\,(aq)}^{-}$ and $CO_{3\,(aq)}^{2-}$ from $\delta^{13}C\text{-}\Sigma CO_2$,[$CO_{2(aq)}$], [$HCO_{3\,(aq)}^{-}$], and [$CO_{3\,(aq)}^{2-}$] data by using the temperature-dependent isotopic enrichment factors reported for the ΣCO_2 system by Zhang et al. (1995). ($\delta^{13}C_{(equilib)}$) was then calculated from the isotopic composition of $HCO_{3\,(aq)}^{-}$ by using the calcite–bicarbonate isotopic enrichment factor reported by Romanek et al. (1992).

3. RESULTS

3.1. Environmental Setting of the Study

Seawater temperature exhibited a typical annual pattern (Fig. 1a) with minimum and maximum temperatures of 6.3 and 19.9°C in February–March and August, respectively. The box-mounted data logger showed water temperatures to be in excellent agreement with those routinely measured at Menai Bridge Pier. $\delta^{18}O\text{-}H_2O$ variations were aperiodic, of low magnitude (Fig. 1a) (with an annual range 0.33‰), and closely correlated with salinity ($\delta^{18}O\text{-}H_2O = -7.42‰ + 0.22S$, $r^2 = 0.9$, $n = 35$.)

Seasonal changes in $\delta^{13}C\text{-}\Sigma CO_2$, the ΣCO_2 system, concentrations of chlorophyll and nutrients were closely influenced by primary production and respiration (Blight et al., 1995). A small diatom bloom at the end of March was followed between mid-April and the end of May by a much larger bloom of diatoms (consisting mainly of the genera *Rhizosolenia* and *Asterionella*). This tailed into a short but intense bloom of the prymnesiophyte *Phaeocystis pouchetti* in early June. At this time, chlorophyll, $\delta^{13}C\text{-}\Sigma CO_2$, pH, and [$CO_3^{2-}$] values reached a maximum, accompanied by a sharp decrease in [ΣCO_2] (Figs. 1b,d). Concentrations of chlorophyll then rapidly decreased in mid-June after extreme nitrate depletion (Fig. 1b) as the *Phaeocystis* bloom crashed, with an accompanying return to pre-bloom values of $\delta^{13}C\text{-}\Sigma CO_2$, [$\Sigma CO_2$], and pH. Changes in $\delta^{13}C\text{-}\Sigma CO_2$ and [ΣCO_2] clearly reflected the processes of dissolved inorganic carbon assimilation by phytoplankton and bacterial respiration and the associated isotope fractionation effects (O'Leary, 1981; Kroopnick, 1985).

3.2. Seasonal Variation in Monthly *Pecten* Shell $\delta^{18}O$ and $\delta^{13}C$

Shell oxygen isotopic data for the monthly scallops are presented in Figure 2a. Between October and mid-June, while the seasonal profile of $\delta^{18}O_{(Pecten)}$ closely tracked $\delta^{18}O_{(equilib)}$, scallop data were more positive than $\delta^{18}O_{(equilib)}$. Between

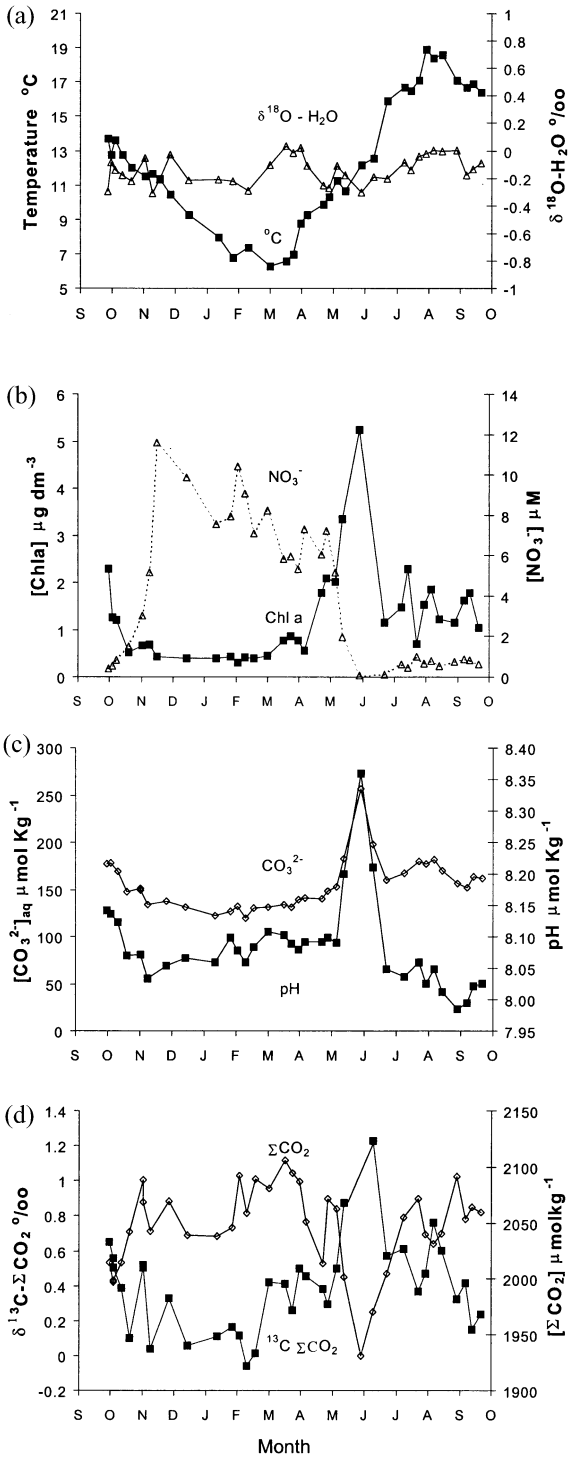

Fig. 1. Seasonal variation in water temperature, $\delta^{18}O\text{-}H_2O$ (VSMOW), chlorophyll *a*, nitrate, pH, $[CO_3^{2-}{}_{(aq)}]$, $\delta^{13}C\text{-}\Sigma CO_2$, and $[\Sigma CO_2]$ at Menai Bridge Pier.

mid-June and September 1995, $\delta^{18}O_{(Pecten)}$ were either in agreement with or more negative than $\delta^{18}O_{(equilib)}$. Shell growth of monthly scallops displayed distinct seasonality (Fig. 3), with growth rates falling to a minimum in January to February (when shell growth effectively ceased), then rising to a maximum in the period June to August before tailing off in September 1995. The effect of shell growth rate on $\delta^{18}O_{(Pecten)}$ is illustrated in Figure 4a. At low shell growth rates, $\delta^{18}O_{(Pecten)}$ were more positive than $\delta^{18}O_{(equilib)}$ values by an average of +0.41 ‰ ($1\sigma = 0.10$‰) at growth rates below 0.13 mm per day. There was a clear negative trend in $\delta^{18}O_{(Pecten)}$ values with increasing shell growth rate. Highest shell growth rates occurred between June and August (Fig. 3), and depletions in $\delta^{18}O_{(Pecten)}$ with respect to $\delta^{18}O_{(equilib)}$ can be observed at this time (Fig. 2a), $\delta^{18}O_{(Pecten)}$ deviating back toward the $\delta^{18}O_{(equilib)}$ line in September when growth rates decreased.

Unlike the shell oxygen isotopic data, $\delta^{13}C_{(Pecten)}$ values for monthly scallops were always more negative than $\delta^{13}C_{(equilib)}$ (Fig. 2b), but as with the $\delta^{18}O_{(Pecten)}$ data, growth rate influenced $\delta^{13}C_{(Pecten)}$ (Fig. 4b), with a trend toward increasingly negative values at higher shell growth rates. The greatest depletions in $\delta^{13}C_{(Pecten)}$ occurred between June and August, associated with the higher shell growth rates at this time.

3.3. Annual Records of *Pecten* Shell $\delta^{18}O$ and $\delta^{13}C$

Shell isotopic profile data for the two annual scallops are shown in Figures 5a,b. For both scallops, $\delta^{18}O_{(Pecten)}$ data are reported relative to the disturbance mark associated with initial deployment in the field. $\delta^{18}O_{(Pecten)}$ data increased to a maximum of 1.84 and 1.97‰ at 7 to 9 mm, then decreased to a minimum of −1.76 and −1.59‰ at 31 −33 mm before rising toward the end of the profile. Both annual scallop shells displayed consistent changes in $\delta^{18}O_{(Pecten)}$ that compared well with the monthly data (Fig. 2a). The only difference between annual and monthly $\delta^{18}O$ profiles was that the annual scallops' isotopic data were telescoped around the $\delta^{18}O$ maximum, reflecting seasonal growth reduction during the period of coldest water temperatures. The consistency between monthly and annual $\delta^{18}O_{(Pecten)}$ data is clearly seen (Table 1) when comparing deviations from equilibrium for annual maximum, minimum, and median $\delta^{18}O_{(Pecten)}$.

As with the $\delta^{18}O_{(Pecten)}$ data, the trend in the $\delta^{13}C_{(Pecten)}$ data was remarkably consistent between both of the annual scallops (Fig. 5b) and when compared with the monthly $\delta^{13}C_{(Pecten)}$ profile (Fig. 2b). The only difference between monthly and annual $\delta^{13}C_{(Pecten)}$ profiles was that two peaks in $\delta^{13}C$ were evident in the monthly profile, whereas three peaks were present in the annual profile. Unlike the $\delta^{18}O_{(Pecten)}$ data, the $\delta^{13}C_{(Pecten)}$ records were offset between the two annual scallops (of the order of 0.2 to 0.4‰ over the profile). This is clearly shown when comparing deviations from predicted equilibrium of maximum, minimum, and median $\delta^{13}C$ data (Table 2). An offset is also evident when comparing annual and monthly $\delta^{13}C$ data (Table 2). A plot of $\delta^{18}O$ against $\delta^{13}C$ (Fig. 6) shows that the *Pecten* isotopic data clearly trend toward more negative values with respect to the predicted equilibrium range of values for the time period after a point 21 mm from the disturbance mark in the shell. By use of average growth rates for each deployment of the monthly scallops and working back from the time of shell recovery in September, it is possible to place a time marker at 21 mm in the shells of the annual scallops of late June. This implies that the most negative isotopic values with respect to predicted equilibria were evident

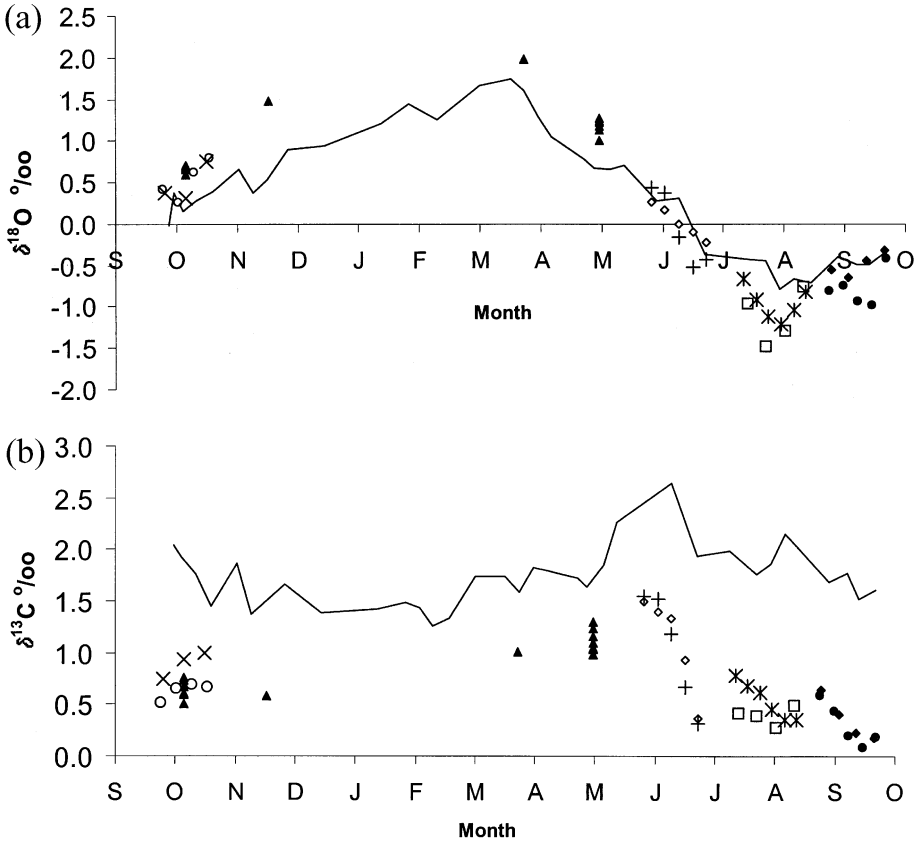

Fig. 2. Seasonal variation in shell $\delta^{18}O$ and $\delta^{13}C$ for scallops placed in the Menai Strait for monthly periods. Each data point represents calcite obtained from an individual scallop. All data reported on the PDB scale. Triangles = data for whole increment grown over the monthly period by scallop. Other markers = profile data from individual scallops for each monthly period. See Methods for details. Solid line = predicted equilibrium (Kim and O'Neil, 1997; 5 mmol kg^{-1} HCO_3^-).

in the summer, associated with the highest shell growth rates in June to September (Fig. 3). A similar trend is evident in a plot of $\delta^{18}O$ against $\delta^{13}C$ for the monthly scallops (Fig. 7), with more negative isotopic values with respect to predicted equilibrium in the period June to August during periods of highest shell growth rate.

Figures 6 and 7 again show that although there is good consistency in the monthly and annual $\delta^{18}O_{(Pecten)}$ data, re-

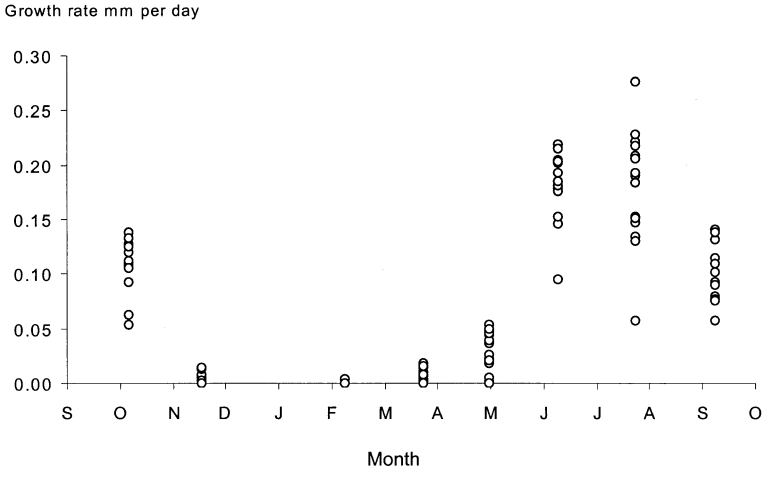

Fig. 3. Seasonal variation in scallop shell growth rates. Each data point is the calculated growth rate from an individual scallop.

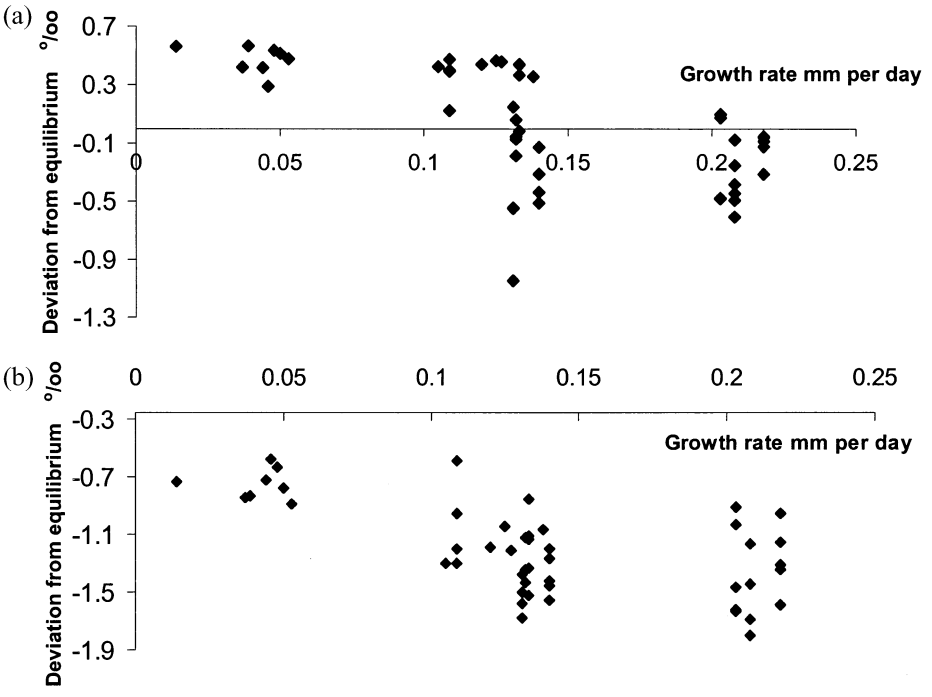

Fig. 4. Deviation in shell $\delta^{18}O$ and $\delta^{13}C$ from predicted equilibrium and its relationship with shell growth rate. All data reported on the PDB scale.

flected by the similar $\delta^{18}O$ distributions, deviations of $\delta^{13}C_{(Pecten)}$ from predicted equilibrium were greater in annual than monthly scallops.

4. DISCUSSION

4.1. Isotopic Behavior at Low Shell-Growth Rates

The results of the field study clearly show the important influence of shell growth rate on *Pecten* $\delta^{18}O$ and $\delta^{13}C$. The enrichments in $\delta^{18}O$ with respect to predicted equilibrium observed at low shell growth rates (of the order of +0.41‰) are comparable to those exhibited by this species at similar growth rates under experimental conditions (Owen et al., 2002). In that study, scallops cultured over the temperature range 10 to 17°C exhibited slow growth rates (<0.1 mm d^{-1}) and enrichments in $\delta^{18}O$ with respect to predicted equilibrium of the order of +0.60‰. The variability associated with the *Pecten* $\delta^{18}O$ data at a given temperature in both experimental (1σ = 0.05 to 0.18 ‰) and field studies (1σ = 0.1‰) was found to be small.

Deviations of shell $\delta^{18}O$ from predicted equilibrium in a number of taxa have been historically explained in terms of a kinetic effect at the CO_2 hydroxylation/hydration steps of the calcification pathway and manifested as depletions with respect to equilibrium (McConnaughey, 1989; McConnaughey et al., 1997). One possible explanation for the enrichments reported here and in the experimental study may lie in differences in the water chemistry between these studies and those in which equilibrium expressions have been established. Experimental and field studies with foraminifera, for example, have shown calcite $\delta^{18}O$ to be influenced by the $[CO_{3\,(aq)}^{2-}]$ of the seawater medium (Spero et al., 1997; Bemis et al., 1998; Russell and Spero, 2000). $\delta^{18}O$–temperature relationships for symbiotic Foraminifera (*Orbulina universa*; Bemis et al., 1998) cultured under low light conditions agreed well with the Kim and O'Neil (1997) expression. We have applied their reported correction factor of −0.002‰ per μmol kg^{-1} $CO_{3\,(aq)}^{2-}$ (a correction factor also calculated by Zeebe, 1999) to the field-collected *Pecten* $\delta^{18}O$ data to account for differences in seasonal $[CO_{3\,(aq)}^{2-}]$ between this study and the experimental data of Bemis et al. (1998). This resulted in insignificant corrections (mean −0.001‰, 1σ = 0.05‰). Similar corrections to the experimental *Pecten* $\delta^{18}O$ data (Owen et al., 2002) to account for differences in $[CO_{3\,(aq)}^{2-}]$ also resulted in only small corrections (+0.082‰, 1σ = 0.015‰). By use of a correction factor derived from symbiont-barren foraminifera of −0.004‰ per μmol kg^{-1} $CO_{3\,(aq)}^{2-}$ (Bijma et al., 1999) also resulted in insignificant corrections (mean +0.003, 1σ = 0.1‰) for the field-collected scallop $\delta^{18}O$ data.

One as yet unexplored possibility is that deviations of scallop $\delta^{18}O$ from predicted equilibrium may reflect a pH effect associated with differences in solution carbonate chemistry at the site of mineralization in the EPF as compared with the external seawater medium. The expression of Kim and O'Neil (1997) is currently the best approximation for equilibrium partitioning of oxygen isotopes between inorganic calcite and bulk seawater, but it is a model that may not be appropriate for isotopic behavior during molluscan calcification. Molluscan biomineralization differs from that of inorganic and foraminiferal calcification in that it occurs within the EPF in an isolated compartment (the extrapallial space) occupying a region between

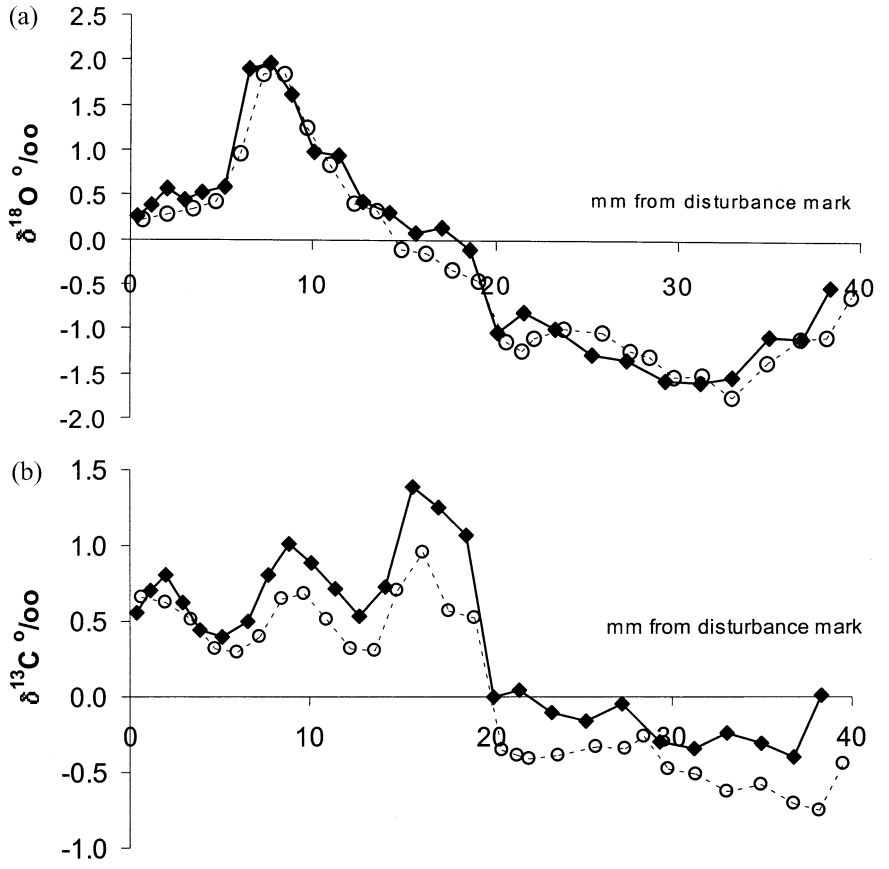

Fig. 5. Profiles of $\delta^{18}O$ and $\delta^{13}C$ for two scallops placed in the Menai Strait for 1 yr. All data reported on the PDB scale. Solid diamonds = scallop A; open circles = scallop B.

the inner mantle epithelium and the outer periostracum (Crenshaw, 1980; Wheeler, 1992). The periostracum itself is hydrophobic and effectively isolates the calcifying environment from the external seawater medium (Crenshaw, 1980), with inorganic carbon supply to the EPF occurring from the hemolymph via the mantle epithelium (Wheeler, 1992). This compartmentalization has been clearly demonstrated in pectinids (Clark, 1974) with marginal calcification (i.e., shell lengthening) occurring under a thin (0.2 μm) periostracum on the upper surface of the mantle. Measurements of bivalve EPF have shown its solution carbonate chemistry to be very different from that of the external seawater environment. ΣCO_2 concentrations have been reported, for example, to be approximately double that of ambient (Crenshaw, 1972) and pH to be substantially lower than that of the external medium for subtidal bivalves in general and scallops in particular (averaging 7.3 to 7.5 as compared with 7.9 to 8.2 for the surrounding seawater; Crenshaw, 1972; Wada and Fujinuki, 1976). As such, the observed enrichments in shell $\delta^{18}O$ may reflect lower pH at the site of mineralization in the EPF as compared with the external seawater medium.

However, studies of EPF pH have focused on an area within the pallial line (an area associated with shell thickening) rather than the marginal EPF (a reflection in part of the inaccessibility of this compartment), and these regions may have very different chemical microenvironments (Crenshaw, 1980; Nair and Robinson, 1998). The hypothesis of a pH-mediated influence on shell $\delta^{18}O$ (perhaps resulting from a kinetic effect at the CO_2 hydroxylation/hydration steps—steps where isotopic equilibration is most sensitive to pH; McConnaughey, 1989; Bijma et al., 1999) can only be confirmed by careful characterization of the ΣCO_2 system in the marginal EPF.

We have pooled the experimental and field *Pecten* $\delta^{18}O$ data at low growth rates (<0.13 mm per day) to derive an expression describing the relationship between scallop shell oxygen

Table 1. Differences between *Pecten* and predicted equilibrium median, maximum, and range of $\delta^{18}O$ data. All data are reported on the PDB scale.

Variable	Δ Median ‰	Δ Maximum ‰	Δ Minimum ‰	Δ Range ‰
Monthly *Pecten*	−0.24	+0.24	−0.71	+0.95
Annual *Pecten* 1	−0.30	+0.22	−0.82	+1.04
Annual *Pecten* 2	−0.44	+0.10	−0.98	+1.08

Table 2. Differences between *Pecten* and predicted equilibrium median, maximum, and range of $\delta^{13}C$ data. All data are reported on the PDB scale.

Variable	Δ Median ‰	Δ Maximum ‰	Δ Minimum ‰	Δ Range ‰
Monthly *Pecten*	−1.07	−1.01	−1.14	+0.12
Annual *Pecten 1*	−1.79	−1.61	−1.97	+0.36
Annual *Pecten 2*	−1.40	−1.18	−1.62	+0.44

isotopes (corrected for the isotopic composition of seawater) and water temperature:

$$\delta^{18}O\text{-}\delta_w = 3.70 - 0.191t°C \quad r^2 = 0.86,\ n = 38,\ p > 0.01.$$

The slope of this relationship agrees well with the inorganic calcite expression of Kim and O'Neil (1997) (−0.200‰ per °C), whereas the intercept is offset by +0.46‰, reflecting the observed enrichments in $\delta^{18}O$.

$\delta^{13}C$ data for *Pecten* grown in the field and exhibiting low growth rates exhibited depletions with respect to predicted equilibrium (up to −2 ‰). Depletions in shell $\delta^{13}C$ (of the order of −2‰) were also found under experimental conditions by Owen et al. (2002) at low shell growth rates for this species. These depletions may be interpreted in terms of a metabolic effect, reflecting introduction of bicarbonate derived from respiratory CO_2 into the EPF, which subsequently becomes incorporated into the shell. Although a causal relationship between metabolic activity and depletion in $\delta^{13}C$ has yet to be convincingly demonstrated, it is generally accepted that such introduction would contribute isotopically depleted carbon into the EPF (Wefer and Berger, 1991; McConnaughey et al., 1997) from which the shell precipitates, masking any potential effect of pH on shell $\delta^{13}C$.

It has been argued (McConnaughey, 1989; Bijma et al., 1999) that because of the action of membrane-bound carbonic anhydrase and the mass-balance effect of oxygen in seawater H_2O, bicarbonate entering the EPF would be in isotopic equilibrium with the external medium with respect to oxygen—that is, such a metabolic effect would be manifested as depletions in $\delta^{13}C$ with respect to equilibrium that are not accompanied by similar depletions in $\delta^{18}O$. Although the $\delta^{13}C$ trend between individuals in the field study is similar, the offset displayed in the annual scallop profiles suggests that the metabolic effect (i.e., respiratory contribution) may be of different magnitude between individuals.

4.2. Isotopic Behavior at Higher Shell-Growth Rates

At higher shell growth rates, there was a clear trend toward increasingly depleted $\delta^{18}O$ values with negative $\delta^{18}O$ values with respect to predicted equilibrium at the highest growth rates. At this time, the magnitude of the $\delta^{13}C$ depletions increased beyond those already observed at slow shell growth rates and deviations in $\delta^{13}C$ from predicted equilibrium varied widely between monthly and annual scallops (Figs. 6 and 7).

However, if, as has been argued above, there is no metabolic modulation of shell $\delta^{18}O$ (McConnaughey, 1989; Bijma et al., 1999), the greater depletions in shell oxygen isotopes with respect to predicted equilibrium observed at higher shell-growth rates may represent a kinetic effect at the CO_2 hydra-

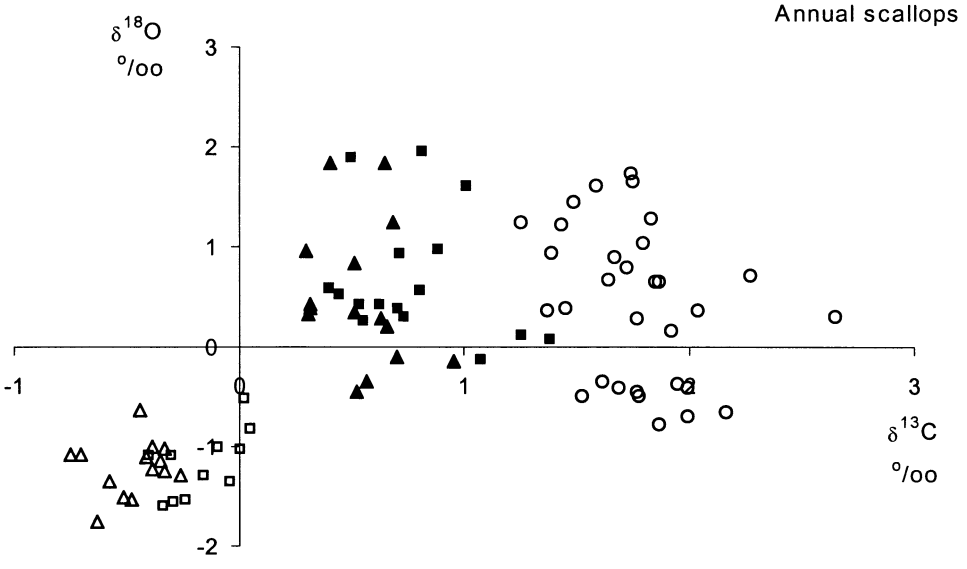

Fig. 6. $\delta^{18}O/\delta^{13}C$ plots for scallops placed in the field for 1 yr. Open circles = predicted equilibrium data, closed triangles and squares = scallop A and B <21mm from disturbance mark, open triangles and squares = scallop A and B >21mm from disturbance mark. All data reported on the PDB scale.

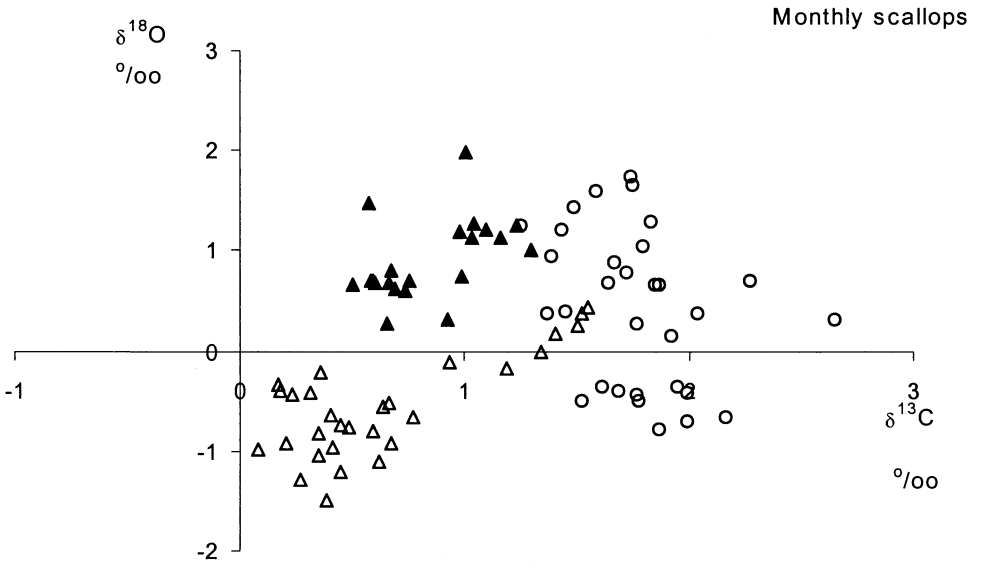

Fig. 7. $\delta^{18}O/\delta^{13}C$ plots for scallops placed in the field for monthly periods. Open circles = predicted equilibrium data; solid triangles = monthly scallops October to June; open triangles = monthly scallops June to September. All data reported on the PDB scale.

tion/hydroxylation steps in the mantle epithelium or $CO_{3\ (aq)}^{2-}$-$CaCO_{3(S)}$ steps in the EPF. Such an effect has been previously suggested as causing simultaneous $\delta^{18}O$ and $\delta^{13}C$ depletions observed in other biogenic carbonates (McConnaughey, 1989; McConnaughey et al., 1997). We suggest that this kinetic effect would be superimposed on a variable metabolic modulation of shell $\delta^{13}C$, resulting in the further depletions in $\delta^{13}C$ observed in addition to those exhibited at low shell growth rates.

There is strong evidence that molluscan calcification is both initiated and regulated via organic matrix constituents of the periostracum and EPF (Wheeler et al., 1981, 1988; Wheeler and Sikes, 1984; Borbas et al., 1991) and that mineralization in the EPF may be associated with active pH control (Wheeler, 1992). Wheeler (1992) cites evidence for a model where CO_2 diffuses from the hemolymph into the mantle epithelial cells and is hydrated or hydroxylated to HCO_3^-, catalyzed by carbonic anhydrase (Crenshaw, 1980). Bicarbonate from this source and that already within the hemolymph may then enter the EPF by active transport mechanisms. H^+ ions generated by net reaction of HCO_3^- and Ca^{2+} may then react with additional HCO_3^- (catalyzed by membrane-bound carbonic anhydrase) to produce CO_2 that diffuses out of the EPF, providing proton buffering within the EPF. Such pH regulation within the EPF may explain the consistency of $\delta^{18}O$ data observed in both laboratory and field studies. However, further work is necessary to accurately characterize the effect of variations both in physiology and shell growth rate on the EPF ΣCO_2 system and isotopic consequences.

We have summarized the data in terms of a hypothetical model of isotopic behavior, as shown in Figure 8. At low shell growth rates, precipitation of shell calcite occurs within the EPF, the solution carbonate chemistry of which differs from the external seawater medium with concurrent influences on shell $\delta^{18}O$. At these low growth rates, there is a metabolic modulation of shell $\delta^{13}C$, which may be variable in magnitude. At higher shell growth rates, a kinetic effect influences both shell $\delta^{18}O$ and $\delta^{13}C$, superimposed on the metabolic influence of shell carbon isotopes.

4.3. Comparison with Previous Studies of Bivalve Shell Isotopes

Most contemporary field studies of molluscan $\delta^{18}O$ and $\delta^{13}C$ (e.g., Killingley and Berger, 1979; Krantz et al., 1984; Margosian et al., 1987; Tan et al., 1988; Barrera et al., 1990; Dare and Deith, 1991; Weidman et al., 1994; Klein et al., 1996a,b; Dettman et al., 1999; Hickson et al., 1999; Leng and Pearce, 1999) have not compared $\delta^{18}O_{(equilib)}/\delta^{13}C_{(equilib)}$ values derived from high-resolution measurements of $\delta^{18}O$-H_2O, water temperature, and the carbon speciation and isotopic composition of the ΣCO_2 system, with seasonal shell isotopic data known to be absolutely contemporaneous with equilibrium data. Comparison of $\delta^{18}O$ and $\delta^{18}O_{(equilib)}$ has also been at times further complicated by seasonal shell growth cessation, which often occurs at cooler water temperatures in many temperate bivalve species (Jones et al., 1983; Dare and Deith, 1991; Weidman et al., 1994; Dettman et al., 1999). However, as reported in this study, studies of marine bivalve shell carbon isotopes (e.g., Killingley and Berger, 1979; Klein et al., 1996b; McConnaughey et al., 1997) have shown marine bivalve $\delta^{13}C$ to be consistently depleted (by on average 2‰ or less) with respect to gross estimated equilibrium values. In addition, negative $\delta^{18}O$ values with respect to predicted equilibrium are evident during periods of summer growth in several studies of temperate bivalve seasonal $\delta^{18}O$ (scallops: Krantz et al., 1984; Tan et al., 1988; Dare and Deith, 1991; mussels: Klein et al., 1996a).

Furthermore, it is interesting to note that in both the Epstein et al. (1953) and Horibe and Oba (1972) studies (the only previous constrained experimental studies, involving mainly

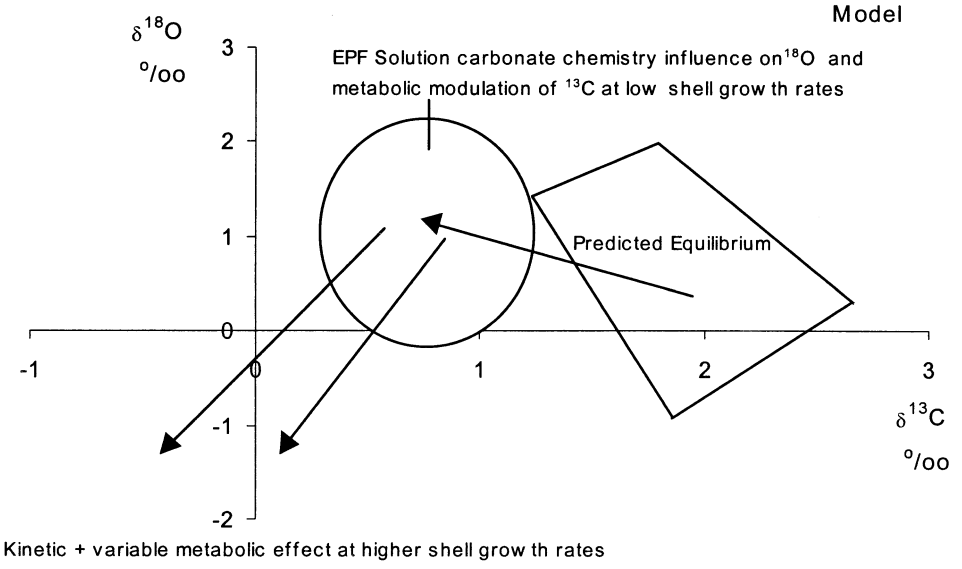

Fig. 8. Model describing the $\delta^{18}O/\delta^{13}C$ plots in Figures 6 and 7.

calcitic molluscs), consistent enrichments in shell $\delta^{18}O$ with respect to the Kim and O'Neil (1997) equilibrium line are also displayed at low temperatures, data approximating or displaying negative values with respect to predicted equilibrium at higher temperatures. This may imply that the model of isotopic behavior postulated here for scallop calcite may not be specific to the species studied here.

5. IMPLICATIONS

The results suggest that accurate and precise high-resolution records of water temperature/$\delta^{18}O$-H_2O may only be obtained from *Pecten* calcite precipitated at low growth rates, although it should be noted that because shell growth effectively ceased below 8 to 9°C, the coldest water temperatures are not recorded in the shell $\delta^{18}O$ record. The combined effects of seasonal growth cessation and a kinetic effect at higher shell growth rates (which occurred at higher water temperatures) had repercussions for determination of the annual median water temperature and its seasonal range from the shell isotopic record (Table 1). *Pecten*-derived median $\delta^{18}O$ was offset from the true median by on average −0.33‰, a temperature equivalency of more than +1.5°C. The *Pecten*-derived annual range of $\delta^{18}O$ was greater than the actual range (by on average 1.02‰), a temperature equivalency of more than 5°C.

However, the scallop $\delta^{18}O$ record is still of great use for identifying broad seasonality in the shell to which shell growth patterns and other aspects of shell chemistry may be related. For example, $\delta^{18}O$ profiles have been used to establish that regions of high abundance of striae (microgrowth lines visible on the scallop shell surface) are deposited during periods of cold water temperature in Winter for *Pecten maximus* in U.K. coastal waters (Dare, 1991). In this way, the use of profiles of striae abundance for routine ageing of scallops and in constructing growth curves has been independently calibrated.

We suggest a combination of both kinetic and variable metabolic effects preclude accurate determination of high resolution $\delta^{13}C$-ΣCO_2 from the scallop shell $\delta^{13}C$ record. However, for other molluscan species, if it can be demonstrated that metabolic effects, kinetic effects, or both are constant at all growth rates and between individuals, there remains the possibility of such $\delta^{13}C$-ΣCO_2 derivations from the shell isotopic record.

We conclude that careful calibration studies are essential for any considered species to allow imposition of constraints on the interpretation of the isotopic record. We also suggest that further studies are required to fully characterize the molluscan marginal EPF ΣCO_2 system under a range of shell growth rates, testing the model of isotopic behavior postulated here for molluscan shell calcite.

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