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Experimental investigation into partitioning of stable isotopes between scallop (*Pecten maximus*) shell calcite and sea water

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Abstract

Stable isotopic compositions of bivalve shells have often been used for the reconstruction of high-resolution records of palaeotemperature and palaeoproductivity cycles. A major assumption in such studies is that isotopic equilibrium between shell carbonate and sea water is maintained at the time of precipitation. This assumption was tested in the laboratory for scallops, *Pecten maximus*, cultured over the temperature range 10–17°C. At the low shell growth rates exhibited (<0.1 mm day^{-1}), deviations of shell $\delta^{18}O$ from equilibrium were +0.6‰ over the experimental temperature range, a temperature equivalency of approximately −3°C. This is hypothesised as reflecting possible differences in the solution carbonate chemistry at the site of mineralisation in the extrapallial fluid (EPF) as compared to that of the external sea water medium, from which the EPF is isolated. Measured depletions of shell $\delta^{13}C$ (of the order of −2.0‰) are interpreted as resulting from introduction of ^{13}C-depleted respiratory CO_2 into the EPF and subsequent incorporation into the shell.

Keywords: Bivalvia; Pectinidae; calcite; stable isotopes; equilibrium

1. Introduction

Both theoretical calculations (Urey, 1947) and constrained experiments (McCrea, 1950; O'Neil et al., 1969; Kim and O'Neil, 1997) have shown that the oxygen isotopic composition (^{18}O:^{16}O) of a slowly precipitating carbonate ($\delta^{18}O$) is governed both by the oxygen isotopic composition of the water ($\delta^{18}O$-H_2O) in which precipitation occurs and the water temperature; isotopic partitioning between the carbonate and water occurring as a result of temperature-dependent equilibrium isotope effects (Urey, 1947). More recently, it has been demonstrated that foraminiferal $\delta^{18}O$ is also influenced by the aqueous carbonate ion concentration, i.e. pH of the medium from which the carbonate precipitates (Spero et al., 1997; Bemis et al., 1998, Bijma et al., 1999).

The carbon isotopic composition of carbonates ($\delta^{13}C$) is governed by the isotopic composition of dissolved inorganic carbon ($\delta^{13}C$-ΣCO_2) and pH of the water from which the carbonate precipitates (Romanek et al., 1992; Spero et al., 1997; Bijma et al., 1999). In oceanic environments major factors influencing $\delta^{13}C$-ΣCO_2 are the process-

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es of primary production and oxidation of organic matter (Kroopnick, 1985).

The potential for establishing high-resolution records of water temperature/$\delta^{18}O$-H_2O and $\delta^{13}C$-ΣCO_2 from $\delta^{18}O$ and $\delta^{13}C$ profiles taken along the axis of molluscan shell growth is well-recognised. Scallops, epifaunal bivalves with an almost entirely calcitic shell, are widely distributed throughout the shallow oceans of the world, are well-represented within the geological record at all latitudes (Barrerra et al., 1990; Smith, 1991; Bice et al., 1996), and are therefore excellent case organisms for palaeoenvironmental reconstruction. Indeed a number of studies have utilised scallop isotopic profiles to establish historical records of water temperature/$\delta^{18}O$-H_2O and $\delta^{13}C$-ΣCO_2 (Krantz et al., 1984, 1987; Tan et al., 1988; Dare and Deith, 1989; Krantz, 1990; Hickson et al., 1999; Johnson et al., 2000).

The establishment of such high-resolution records is based upon the hypothesis that isotopic equilibrium was established between shell carbonate and sea water at the time of precipitation. Several processes have been suggested which may result in deviations of molluscan shell isotopes from equilibrium. A metabolic effect may result in introduction of isotopically depleted respiratory CO_2 into the shell. Alternatively, or in addition to this, a kinetic effect may occur, perhaps as a result of incomplete isotopic partitioning between $CO_{2(aq)}$ and $HCO^-_{3(aq)}$ at the CO_2 hydroxylation/hydration steps or CO^{2-}_3(aq)-$CaCO_{3(s)}$ steps in the biomineralisation pathway (McConnaughey, 1989; McConnaughey et al., 1997; Bijma et al., 1999).

The maintenance of isotopic equilibrium between biologically precipitated carbonates and sea water has historically been established in two ways. Firstly, isotopic data obtained from individuals collected in the field have been compared with predicted $\delta^{18}O$ and $\delta^{13}C$ values for carbonate precipitated in isotopic equilibrium with sea water. Such predicted values are calculated from temperature, $\delta^{18}O$-H_2O and $\delta^{13}C$-ΣCO_2 data collected from the environment in which the organism grew (Krantz et al., 1984; Margosian et al., 1987; Tan et al., 1988; Dare and Deith, 1989; Weidman et al., 1994; Klein et al., 1996a,b; Hickson et al., 1999). Secondly, a number of laboratory studies have investigated partitioning of stable isotopes between biogenic carbonates and sea water for molluscs (Epstein et al., 1953; Horibe and Oba, 1972), coccolithophorids (Dudley and Goodney, 1979), ostracods (Xia et al., 1997) and foraminifera (Erez and Luz, 1983; Spero and Williams, 1988; Spero, 1992; Spero and Lea, 1993, 1996; Spero et al., 1997; Bemis et al., 1998). However, not a single laboratory study has investigated the maintenance of oxygen isotopic equilibrium between molluscan shell carbonate and sea water for a given species over its natural temperature range under constrained conditions whereby all the governing factors (temperature, solution chemistry, $\delta^{18}O$-H_2O and $\delta^{13}C$-ΣCO_2) have been simultaneously measured. Additionally, laboratory studies considering the partitioning of carbon isotopes between molluscan shell carbonate and dissolved inorganic carbon have been restricted to the one study of Fritz and Poplawski (1974), for a number of fresh water molluscs.

In this study we investigate by laboratory experiment the partitioning of oxygen and carbon isotopes between scallop, *Pecten maximus*, shell calcite and sea water under constrained conditions, evaluating the use of this scallop in contemporary and palaeoenvironmental reconstruction.

2. Methods

2.1. Culturing of scallops for $\delta^{18}O$ and $\delta^{13}C$ determination

Juvenile *Pecten maximus*, approximately 8 months old and measuring 15–20 mm shell height (umbo to valve margin), and comprising a cohort from a single spat settlement, were placed in four insulated tanks containing re-circulating sea water (85 l per tank) with temperature regulation to +/−0.1°C. Scallops were acclimated from ambient water temperature to one of the four experimental temperatures (10, 13, 15 and 17°C, in tanks 1–4 respectively). Experimental temperatures were selected to take into account the fact that shell deposition is believed to cease below 8–9°C (Mason,

1957) but encompassing the upper range of sea water temperature experienced by scallops in the shelf seas around the UK. After acclimation each scallop was removed and carefully marked along the shell valve margin using an indelible pen and then allowed to grow for 1 month at the experimental temperature. Scallops in each tank were fed daily a mixed algal culture containing predominantly *Pavlova lutherii* and *Skeletonema costatum*. A small flux of mains-supplied sea water was maintained through each tank without compromising temperature constancy. The latter was monitored by rotating a tank-mounted data logger (Tiny Talk Temperature Logger, Orion Instruments, Chichester, UK) every 7 days between the tanks to assess temperature stability, whilst a temperature probe in tank 1 attached to a Rustrak Chart Recorder continuously monitored the temperature for the experimental duration. After 38 days scallops were removed from the tanks and the entire increment of shell precipitated during the experiment (i.e. between the indelible pen mark and the valve margin) carefully removed using a dentists drill. Powdered shell calcite from each individual was collected into a separate glass tube and pretreated to remove organic material in a low temperature oxygen plasma for 4.5 h (Swart, 1981). Calcite samples were reacted with 2.5 cm^3, 104% H_3PO_4 at 25 ± 0.1°C (McCrea, 1950). All stable isotopic analyses were performed on a VG SIRA series II dual inlet isotopic ratio mass spectrometer (IRMS). For calibration, replicate CO_2 samples from an acidified accredited carbonate standard (NBS19) were run against the IRMS reference gas CO_2 to determine the isotopic composition of this gas relative to NBS19. The isotopic composition of the sample CO_2 prepared from acidified, pretreated shell was then determined using this and the reported isotopic composition of NBS19 relative to PDB (Craig, 1957). Calcite oxygen isotopic ratios ($\delta^{18}O$) were then calculated using an acid fractionation factor of 1.01025 (Friedman and O'Neil, 1977) and reported on the PDB scale. Precisions associated with calcite isotopic data as determined from replicate analyses of inorganic marble chips were $1\sigma = 0.08$‰ for $\delta^{18}O$ and $1\sigma = 0.04$‰ for $\delta^{13}C$.

2.2. Sea water analyses for determination of isotopic data for calcite precipitated in isotopic equilibrium with sea water

Water samples were collected from each tank at regular intervals over the experiment for salinity, $\delta^{18}O$-H_2O, concentration of dissolved inorganic carbon ($[\Sigma CO_2]$), $\delta^{13}C$-ΣCO_2 and pH determination. Salinity samples were analysed with an AutoSal 8400 Autosalinometer calibrated with I.A.P.S.O. sea water (precision of replicates $1\sigma = 0.0004$ S). Sea water samples were analysed for $\delta^{18}O$-H_2O by sealed vessel CO_2 equilibration (Epstein and Mayeda, 1953; Frew et al., 1995), precision of replicates $1\sigma = 0.04$‰. Sample water oxygen isotopic compositions were initially reported relative to VSMOW ($\delta^{18}O$-H_2O). The method involved equilibrating 2.5 cm^3 of the sample with CO_2 at 25 ± 0.1°C for 48 h. The oxygen isotopic composition of this CO_2 was determined relative to a reference water sample (North Sea Water (NSW), +0.197‰ relative to VSMOW, Frew et al., 1995). NSW was run directly against VSMOW in our laboratory using the same method with a measured value of +0.175‰. Samples were routinely run in batches alongside NSW, expressed relative to NSW and thereby relative to VSMOW. A secondary reference water sample (Norwich Tap Water, −6.836‰ relative to VSMOW) was run within each sample batch to check for accuracy.

$[\Sigma CO_2]$ and $\delta^{13}C$-ΣCO_2 were determined from samples of water that had been siphoned from each tank into a glass-stoppered bottle and poisoned immediately with saturated $HgCl_2$ at 1 cm^3 dm^{-3} to halt biological activity (Kroopnick, 1980). Within 2 h a 10-cm^3 subsample was filtered (0.4 μm Nuclepore) into a pre-weighed, nitrogen-flushed ampoule (Adelphi Tubes, UK), flame-sealed and the ampoule re-weighed. The ampoule was subsequently cracked under vacuum and acidified with H_3PO_4 for quantitative CO_2 extraction from the water sample. The evolved CO_2 was scrubbed of water vapour and non-condensible gases using similar procedures outlined in Kroopnick (1974) and McCorkle (1987). The number of moles of CO_2 was determined by an on-line manometer, which, along with the sample weight de-

termined above, allowed determination of [ΣCO_2] (precision of replicates $1\sigma = 17.5$ μmol kg^{-1}). The CO_2 was then quantitatively transferred to a collection vessel for isotopic determination (precision of replicates $1\sigma = 0.03$‰). pH was measured spectrophotometrically in a 10-cm optical cell using *m*-cresol purple (Clayton and Byrne, 1993). The extinction at 436, 578 and 730 nm was measured simultaneously in a Hewlett Packard diode array spectrophotometer (precision of replicates $1\sigma = 0.0067$ μmol kg^{-1}). The measured pH, temperature at time of pH measurement, associated [ΣCO_2] and salinity for the water sample as determined above were used to calculate the total alkalinity within the optical cell. Total alkalinity, salinity, [ΣCO_2] and in situ tank temperature were then used to determine the concentration of dissolved inorganic carbon species in the tank.

The predicted $\delta^{18}O$ of calcite precipitated in isotopic equilibrium with sea water (corrected for the oxygen isotopic composition of sea water) was determined using the most recent data for inorganic calcite–water oxygen isotope exchange reported by Kim and O'Neil (1997). To allow comparison with data in the literature, calcite and water oxygen isotopic data reported against SMOW by Kim and O'Neil (1997) were converted to the PDB scale. Calcite oxygen isotopic data reported on the SMOW scale were corrected (+0.25‰) to account for differences in the acid fractionation factors used in the studies (1.01050 c.f. 1.01025) and then converted to the PDB scale using the following equation:

$$\delta^{18}O\ (PDB) = 0.97006\ \delta^{18}O\ (SMOW) - 29.94$$

(Friedman and O'Neil, 1977)

Similarly, $\delta^{18}O$-H_2O data reported by Kim and O'Neil (1997) on the SMOW scale were 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), thereby allowing comparison with earlier studies:

$$\delta_w = 0.99978\ \delta^{18}O\ (SMOW) - 0.22$$

(Friedman and O'Neil, 1977)

These conversions use a CO_2-calcite fractionation factor associated with sealed vessel acidification at 25°C of 1.01025 and CO_2-H_2O fractionation factor at 25°C of 1.0412 (Friedman and O'Neil, 1977). A linear regression of the Kim and O'Neil (1997) calcite $\delta^{18}O$ data, corrected for δ_w, against water temperature was then used to calculate the predicted ($\delta^{18}O$-δ_w)/temperature relationship for inorganic calcite precipitated in isotopic equilibrium with sea water. Bemis et al. (1998) show the appropriateness of using linear versus quadratic temperature/$\delta^{18}O$ relationships at warm oceanic temperatures (9–24°C). To allow direct comparison, *Pecten* $\delta^{18}O$ data were corrected for δ_w at each temperature, using mean δ_w data for each tank over the experimental period. The latter was converted from the SMOW to PDB scales as above.

The carbon isotopic composition of calcite precipitated in isotopic equilibrium with sea water in each tank ($\delta^{13}C_{equilib}$) was determined from a mass balance equation relating $\delta^{13}C$-ΣCO_2 to the concentrations and isotopic compositions of $CO_{2(aq)}$, $HCO^-_{3(aq)}$ and CO^{2-}_3(aq) and 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 $HCO^-_{3(aq)}$, using the calcite–bicarbonate isotopic enrichment factor reported by Romanek et al. (1992).

3. Results

3.1. Partitioning of oxygen isotopes between Pecten shell calcite and sea water

Water temperature was effectively constant within each tank throughout the experiment ($1\sigma = 0.06$–0.08°C). Salinity variation within each tank was small ($1\sigma = 0.07$ S) and analysis of variance showed there to be no significant differences in mean salinities between tanks over the duration of the experiment ($F = 0.60$, $P = 0.621$, d.f. = 31). $\delta^{18}O$-H_2O data are shown in Table 1. The variability in $\delta^{18}O$-H_2O within each tank for the experimental duration was low ($1\sigma = 0.04$–0.06‰) and close to the analytical error. Analysis of var-

Table 1
Experimental $\delta^{18}O$-H_2O data (‰ relative to VSMOW)

Day	9.9°C tank	12.9°C tank	14.7°C tank	19.9°C tank
6	0.05	0.03	0.12	0.04
15	0.12	0.16	0.08	0.10
23	0.09	0.11	0.05	0.07
27	−0.05	0.07	−0.00	0.03
34	0.05	0.11	0.08	−0.04

iance showed there to be no significant difference in $\delta^{18}O$-H_2O between the four tanks over the experimental period ($F = 1.07$, $P = 0.391$, d.f. = 19).

Partitioning of oxygen isotopes between *Pecten* shell calcite and sea water as shown in Fig. 1 was well-described by the linear relationship:

$$\delta^{18}O_{(Pecten)} - \delta_w = 3.85 - 0.196\ (^{\circ}C)$$

$$(n = 22,\ r^2 = 0.91,\ P = <0.01)$$

The slope derived from the experimental data compares favourably with the slope of −0.200‰ for the $\delta^{18}O$-δ_w/temperature relationship reported by Kim and O'Neil (1997) at 5 mmol kg^{-1} HCO_3^-. However, whilst there is a well-constrained temperature dependency associated with the *Pecten* calcite–water fractionation factor, *Pecten* data lie above the equilibrium line by +0.6‰ over the experimental temperature range. The variability associated with $\delta^{18}O_{(Pecten)}$ at any one temperature was of the order $1\sigma = 0.05$–0.18‰. This is similar to the variability associated with the Epstein et al. (1953) molluscan

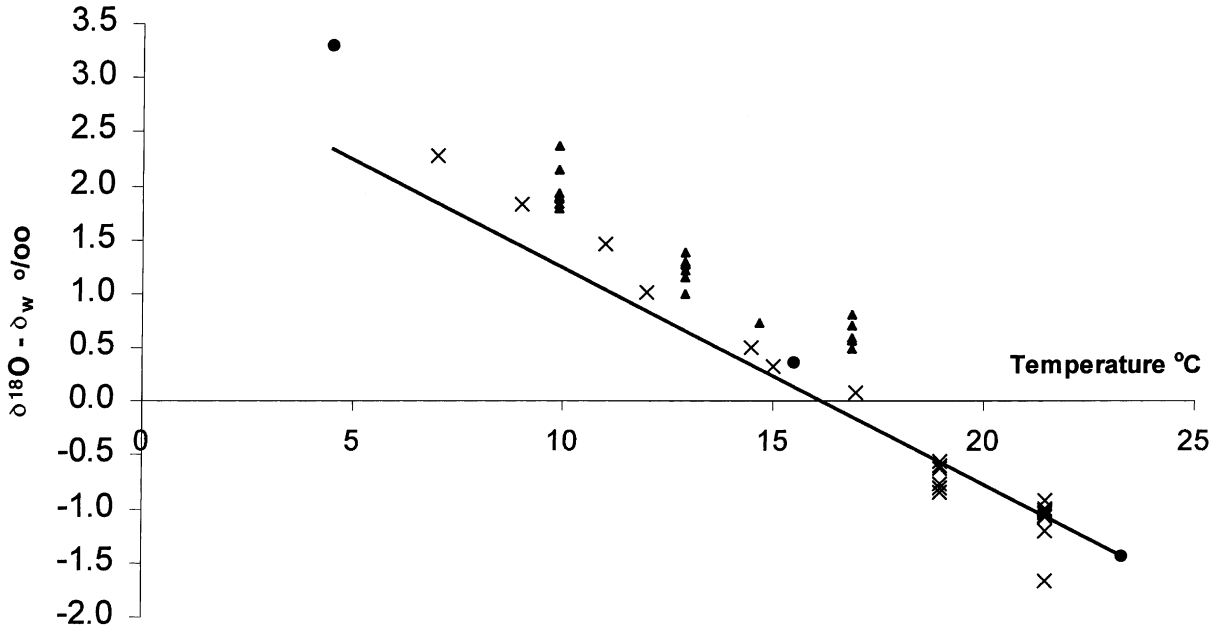

Fig. 1. Plots of $\delta^{18}O_{(equilib)}$-δ_w (calculated using the Kim and O'Neil (1997) expression at 5 mmol kg^{-1} HCO_3^-) and $\delta^{18}O_{(Pecten)}$-δ_w against water temperature. All data on the PDB scale. Also shown for comparison are the Epstein et al. (1953) and Horibe and Oba (1972) data. Each data point from this study represents calcite deposited by an individual scallop over the experimental duration. Legend: ▲ *Pecten maximus*; – Kim and O'Neil (1997) (5 mmol kg^{-1} HCO_3^-); × Epstein et al. (1953); ● Horibe and Oba (1972).

Table 2
Experimental $\delta^{13}C$-ΣCO_2 data (‰ relative to vPDB)

Date	9.9°C tank	12.9°C tank	14.7°C tank	19.9°C tank
6	−2.01	−4.53	−0.12	0.09
10	−0.82	−0.68	−3.90	−4.21
13	0.08	0.17	−1.52	−1.70
15	−0.35	0.18	−1.37	−1.76
17	0.14	0.18	0.00	−0.19
23	−0.01	0.09	−0.44	−0.81
27	0.37	0.37	−2.21	−3.23
34	0.00	0.14	0.31	0.20

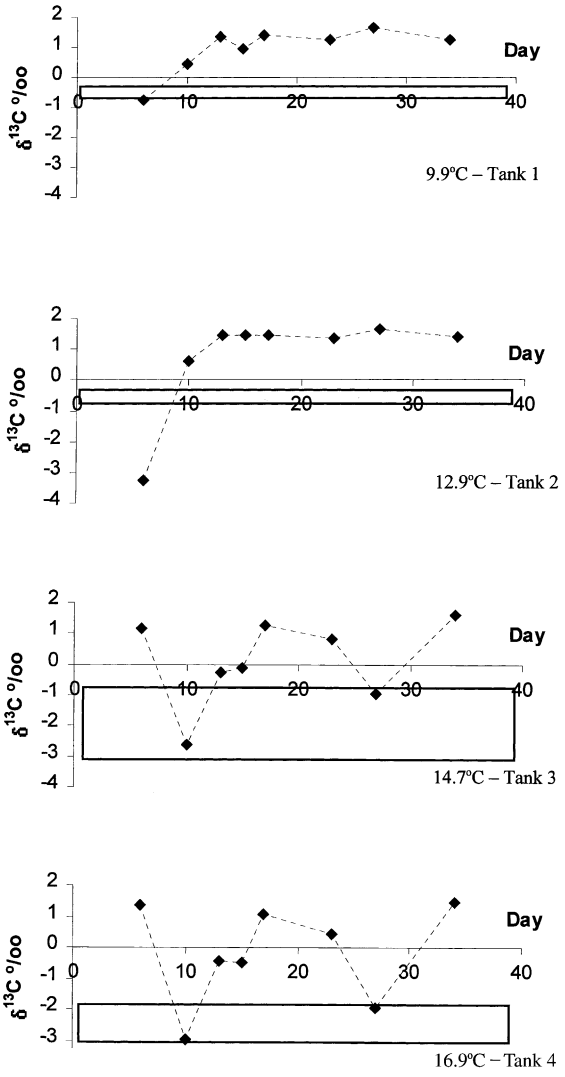

Fig. 2. Experimental $\delta^{13}C_{(equilib)}$ and $\delta^{13}C_{(Pecten)}$ data. Horizontal bar shows range of $\delta^{13}C_{(Pecten)}$ data. All data on the PDB scale. Legend: ♦ $\delta^{13}C_{(equilib)}$.

data (0.11–0.22 ‰) and is marginally higher than the expected (methodological) variability of 0.1 ‰. The latter was calculated by propagation of errors associated with carbonate $\delta^{18}O$ determination and within tank variation in water temperature and δ_w.

3.2. Partitioning of carbon isotopes between Pecten shell calcite and sea water

$\delta^{13}C$-ΣCO_2 was not constant during the course of the experiment at any of the temperatures (Table 2, Fig. 2). In tanks 1 and 2, $\delta^{13}C$-ΣCO_2 was relatively stable after an initial period of isotopically depleted ratios. In the case of tanks 3 and 4 isotopically depleted values were recorded twice during the course of the experiment. Mains-supplied sea water was piped to tanks 1 and 2 via a common manifold and T piece. Tanks 3 and 4 also shared a common supply. It is possible that synchronous changes in water residence time in each pair of tanks due to variations in mains sea water supply, combined with efflux of isotopi-

Table 3
Experimental *Pecten* $\delta^{18}O$ data (‰ relative to vPDB)

9.9°C tank	12.9°C tank	14.7°C tank	19.9°C tank
1.98	1.03	0.94	−0.23
1.76	1.14	0.31	0.21
1.63	1.17	0.58	0.38
1.71	0.87	0.52	0.41
1.72	1.16		0.30
2.19	1.17		0.63
1.66	1.09		0.52
1.90	1.26		−0.23
1.71			0.27
			0.11

Table 4
Experimental *Pecten* $\delta^{13}C$ data (‰ relative to vPDB)

9.9°C tank	12.9°C tank	14.7°C tank	19.9°C tank
−0.53	−0.51	−2.13	−2.59
−0.45	−0.59	−3.11	−3.04
−0.61	−0.38	−1.78	−2.27
−0.49	−0.41	−0.81	−1.97
−0.63	−0.53		−2.31
−0.63	−0.61		−1.90
−0.48	−0.41		−2.79
−0.67	−0.72		−2.34
−0.38			−2.92

cally depleted respiratory CO_2 from the growing scallops or bacteria may have resulted in the excursions in $\delta^{13}C$-ΣCO_2 observed during the experiment. $\delta^{13}C_{(Pecten)}$ were well-constrained in tanks 1 and 2, but showed more variability in tanks 3 and 4 (Tables 3 and 4). We employed appropriate two sample significance tests to test for differences between $\delta^{13}C_{(equilib)}$ and $\delta^{13}C_{(Pecten)}$ data sets at each temperature (Table 5). Where either of the data sets were not normal a Mood test was employed. Where data were normal but did not show equal variance (*F*-test) an unpooled *t*-test was used. Where data were both normal and exhibited equal variances a pooled *t*-test was used.

Table 5 shows that at all temperatures $\delta^{13}C_{(Pecten)}$ data were significantly different from $\delta^{13}C_{(equilib)}$, being isotopically depleted in all cases, despite the observed variability in $\delta^{13}C$-ΣCO_2. Deviations of $\delta^{13}C_{(Pecten)}$ from isotopic equilibrium, i.e. $\delta^{13}C_{(Pecten)}$-$\delta^{13}C_{(equilib)}$, were of the order of −2.02‰ ($1\sigma = 0.20$‰) over the experimental temperature range.

4. Discussion

The negative deviations with respect to equilibrium exhibited by the *Pecten* $\delta^{13}C$ data are in agreement with the findings of McConnaughey et al. (1997) who, in a review of the literature, concluded that molluscan shell $\delta^{13}C$ deviations from equilibrium are typically −2‰ or less. The calculation of $\delta^{13}C_{(equilib)}$ relies on the utilisation of available isotopic enrichment factors for the ΣCO_2 system as determined by Zhang et al. (1995) for $CO_{2(aq)}$, $HCO^-_{3(aq)}$ and $CO^{2-}_{3(aq)}$ in a deionised water matrix. There is some question of the applicability of using the enrichment factor between $CO^{2-}_{3(aq)}$ and CO_2 gas as reported by these authors within the sea water ΣCO_2 system since sea water contains a number of additional carbonate species, notably $MgCO_3$ and $CaCO_3$ (Stumm and Morgan, 1981). This may influence the fractionation between $\Sigma CO^{2-}_{3(aq)}$ (i.e. $CO^{2-}_{3(aq)}$+$CO^{2-}_{3(mineral)}$) and CO_2 gas. Crudely correcting the above enrichment factor by assuming sea water contains 30% aqueous and 70% mineral carbonate and using the carbonate–CO_2 gas enrichment factors reported by Romanek et al. (1992) and Zhang et al. (1995) results in calculated values of $\delta^{13}C_{(Pecten)}$-$\delta^{13}C_{(equilib)}$ of −1.85‰. These are only slightly enriched as compared to those previously calculated.

Biomineralisation within bivalves takes place within the extrapallial fluid (EPF), a compartment bound by the accreting shell/periostracum and mantle epithelium. Inorganic carbon supply to the EPF takes the form of CO_2, which diffuses across the mantle epithelium from the mantle cavity (which contains the same medium as the external sea water for species not undergoing periods of prolonged valve closure), or HCO^-_3, which is actively transported across the mantle epithelium (Wheeler, 1992). In a model of isotopic disequilibrium hypothesised by McConnaughey (1989), deviations of isotopes in biologically precipitated carbonates from equilibrium have been

Table 5
Summary of significance tests between experimental $\delta^{13}C_{(Pecten)}$ and $\delta^{13}C_{(equilib)}$ data sets

	Experimental Temperature			
	9.9°C (tank 1)	12.9°C (tank 2)	14.7°C (tank 3)	16.9°C (tank 4)
Test	Mood	Mood	Pooled T	Non-pooled T
Significance	sig. at $P = < 0.01$	sig. at $P = < 0.01$	sig. at $P = < 0.01$	sig. at $P = < 0.01$
$\delta^{13}C_{(Pecten)}$-$\delta^{13}C_{(equilib)}$	−1.80‰	−1.95‰	−2.07‰	−2.27‰

interpreted as reflecting one of two processes that may act in unison or synergistically. Observed simultaneous deviations in shell $\delta^{18}O$ and $\delta^{13}C$ have been interpreted as a kinetic effect reflecting incomplete isotopic partitioning at the CO_2 hydration/hydroxylation step in the EPF, often occurring at high shell growth rates (this should not be confused with precipitation rate effects in inorganic non-compartmented systems, which have been shown to be negligible, Kim and O'Neil, 1997). The measured enrichments in shell $\delta^{18}O$ with respect to equilibrium observed here do not support the interpretation of the data as reflecting a kinetic effect. In the model, McConnaughey (1989) suggested that measured depletions in shell $\delta^{13}C$ which are not accompanied by simultaneous deviations in shell $\delta^{18}O$ reflect a metabolic effect in which introduction of respiratory CO_2 results in observed depletions in shell $\delta^{13}C$. Unlike carbon isotopes, it has been argued (McConnaughey, 1989; Bijma et al., 1999) that the action of carbonic anhydrase and the relatively small proportion that oxygen in CO_2 comprises of the total oxygen pool (this being largely H_2O) lead to isotopic equilibration with respect to oxygen of the CO_2 entering the EPF with sea water. Thus the most plausible explanation for the observed depletions in shell $\delta^{13}C$ is incorporation of respiratory CO_2 which is isotopically depleted with respect to carbon. This interpretation is in agreement with the general consensus of a metabolic modulation of shell $\delta^{13}C$ (Wefer and Berger, 1991; McConnaughey et al., 1997).

Chemical evidence supports the presence of respiratory CO_2 within the EPF, as derived from pH and dissolved inorganic carbon measurements of that fluid. Molluscan EPF has a pH which is lower than the surrounding medium (7.3–7.6 for bivalves as compared to approximately 8.0 for the medium) and ΣCO_2 concentration approximately double that of ambient (Crenshaw, 1972). However, there is no similar direct measurement of the isotopic composition of molluscan EPF and currently the observed isotopic changes can only be considered as being consistent with the expected trends for the addition and incorporation of metabolic CO_2. The degree to which skeletal $\delta^{13}C$ deviates from equilibrium should be influenced by the animal's metabolic activity. Temperature, food availability, growth and valve gape/closure might all cause species-specific and/or seasonal changes in $\delta^{13}C$ due to the changing influence of metabolic CO_2 on the chemistry and isotopic composition of the EPF. Currently there are little published data that find anything other than gross differences resulting from a relationship between environmental or physiological variables and skeletal $\delta^{13}C$ (Klein et al., 1996b; McConnaughey et al., 1997). Measured deviations of shell $\delta^{13}C$ from equilibrium in our experiment showed no significant trend with temperature (although this may be masked by the variability associated with $\delta^{13}C$-ΣCO_2 within tanks over the course of the study). We emphasise that an assumption underlying the interpretation of the shell carbon isotopic data is that shell growth was continuous throughout the duration of the experiment. However, it should be noted that shell $\delta^{13}C$ data were nearly always depleted with respect to predicted $\delta^{13}C_{equilib}$ values (Fig. 2) and it is unlikely that shell growth occurred exclusively around day 5 in tanks 1 and 2, and day 10 in tanks 3 and 4, periods when shell $\delta^{13}C$ data were not depleted with respect to predicted equilibrium. More likely is uncertainty in the magnitude of the calculated disequilibrium and this should be further investigated.

Some 50 yr since the initial experimental work of McCrea (1950) and Epstein et al. (1953) there still remains some difficulty in reaching a consensus for the expression describing partitioning of oxygen isotopes between calcite and water at equilibrium (Kim and O'Neil, 1997; Bemis et al., 1998; Zeebe, 1999). We accept here the arguments presented by Kim and O'Neil (1997) that justify their data as approximating the equilibrium state at sea water pH. *Pecten* $\delta^{18}O$ were enriched with respect to equilibrium over the experimental temperature range by +0.6‰, a temperature equivalence of −3°C. Despite this enrichment, the slope of the $\delta^{18}O_{(Pecten)}$-δ_w/temperature relationship (−0.196‰ °C^{-1}) agreed well with that of Kim and O'Neil (1997), (−0.200‰ °C^{-1}). Shell $\delta^{18}O$ data reported by Epstein et al. (1953) for molluscs of largely calcitic mineralogy and Horibe and Oba (1972) for the

scallop *Patinopecten yessoensis* also show that whilst the data were in good agreement with equilibrium values at higher temperatures, enrichments with respect to equilibrium also occur at the lower temperature range (Fig. 1). It should be noted that we have used a conversion factor of −0.22‰ for calculating δ_w on the PDB scale from $\delta^{18}O$-H_2O as reported on the SMOW scale (Friedman and O'Neil, 1977). Using a conversion factor of −0.27‰ (Hut, 1987) would result in both the *Pecten* and Kim and O'Neil (1997) $\delta^{18}O$-δ_w data being offset relative to the Epstein et al. (1953) data as graphed in Fig. 1 by +0.05‰, within the analytical error.

The reason for the measured oxygen isotopic enrichment cannot be determined within the existing data set but is not explained in terms of differences in sea water chemistry between this study and those where cultured foraminiferal $\delta^{18}O$ were found to be in agreement with predicted equilibrium data. Experimental studies have shown for example that foraminiferal calcite $\delta^{18}O$ is influenced by the pH of the medium in which the organisms were grown (Spero et al., 1997; Bemis et al., 1998). Symbiotic foraminifera (*Orbulina universa*) cultured under low light conditions at $[CO_3^{2-}]$ of 171 μmol kg^{-1} by Bemis et al. (1998) precipitated tests which agreed well with the 5-$mmol^{-1}$ kg HCO_3^- equilibrium expression of Kim and O'Neil (1997). Given the experimental $[CO_3^{2-}]$ in our study (123–140 μmol kg^{-1} between tanks, $1\sigma = 7$–11 μmol kg^{-1} within tanks) and the correction factor reported by Bemis et al. (1998) of −0.002‰ μmol^{-1} kg^{-1} $[CO_3^{2-}]$, only small applied corrections to the *Pecten* $\delta^{18}O$ data (−0.06 to −0.1‰) would account for differences in sea water $[CO_3^{2-}]$ between the studies. While there is as yet no direct evidence, it is feasible that the measured enrichments of *Pecten* oxygen isotopes with respect to equilibrium may reflect a pH effect resulting from differences in solution carbonate chemistry between the precipitation microenvironment in the EPF and external sea water. As discussed above, there is evidence to suggest that this environment may be quite different: studies of bivalve EPF have reported ΣCO_2 concentrations to be approximately double that of the external sea water medium and pHs which are lower than that of ambient (Crenshaw, 1972). Wada and Fujinuki (1976) found for example that EPF pH for the scallop *Chlamys nobilis* was 7.5 as compared to 8.2 for the external medium.

However, prediction of isotopic behaviour based on the measurement of chemical properties of the bulk EPF may not be appropriate as the solution chemistry at the actual site of mineralisation may differ from that of the surrounding EPF. Studies to date have focussed on the region within the pallial line, rather than the marginal EPF where shell lengthening occurs (Wheeler, 1992), in part reflecting the inaccessibility of the marginal EPF. However, mineralisation at the fluid–shell interface is an organic matrix-mediated process that is associated with active pH control. Reviewing the literature for molluscan mineralisation Wheeler (1992) has given evidence supporting a model whereby net reaction of bicarbonate with calcium generates both $CaCO_3$ and H^+ at the site of mineralisation. Proton buffering and pH regulation in the surrounding EPF then occurs by reaction of the H^+ ions with EPF bicarbonate, catalysed by membrane-bound carbonic anhydrase. It is possible that pH at the site of mineralisation may be lower than that of the bulk EPF and such differences in solution chemistry in the precipitation microenvironment as compared to the external medium may be manifested as the observed enrichments in shell $\delta^{18}O$.

Although *Pecten* shell oxygen isotopes were not precipitated in isotopic equilibrium with sea water, the well-constrained temperature dependency associated with *Pecten* shell calcite–water oxygen isotopic exchange suggests that precise (1 S.D. = 0.25–0.9°C) palaeowater temperature/$\delta^{18}O$-H_2O derivations are still possible using the amended equation we have derived from the experimental data set. Similarly, whilst we suggest a metabolic modulation of shell $\delta^{13}C$, we were unable to resolve any variation in *Pecten* shell $\delta^{13}C$ with temperature, although this may be masked by the variability in experimental ΣCO_2: if it can be demonstrated that this potential metabolic effect is constant there remains the possibility of $\delta^{13}C$-ΣCO_2 derivations from the scallop shell isotopic record.

However, physiological changes such as metabolic rate may control the chemical and carbon isotopic composition of the EPF and hence skeletal $\delta^{13}C$, although the necessary measurements within the EPF to illustrate this have yet to be made. Furthermore, *Pecten* shell growth rates, as determined from measurements of the increment of shell grown by scallops over the experimental duration, were low (0.05–0.1 mm per day), probably reflecting sub-optimal growth conditions under laboratory conditions. Shell growth rates in the Summer for scallops around the UK (at bottom water temperatures of 14°C) have been reported as being much higher (of the order of 0.2 mm per day for juvenile scallops aged 0–2 yr, Mason (1957)). At higher growth rates it has been suggested that there may be a variable contribution of respiratory CO_2 to the EPF and/or incomplete partitioning of oxygen isotopes at the CO_2 hydration/hydroxylation or $CO_3^{2-}(aq)$-$CaCO_{3(s)}$ steps in the biomineralisation pathway (McConnaughey, 1989; McConnaughey et al., 1997; Bijma et al., 1999). The influences of variation in organismal metabolic rate and shell growth rate on shell isotopes should be investigated to address these possibilities.

5. Conclusions

Pecten shell oxygen isotopes exhibited enrichments of +0.6‰ relative to equilibrium which were reproducible across the temperature range studied. Differences between palaeotemperature equations derived from *Pecten* data and those derived from inorganic and foraminiferal calcites may reflect the compartmentalisation of molluscan biomineralisation and associated differences in solution carbonate chemistry at the site of mineralisation in the EPF as compared to the external medium. Further research (in particular characterising the ΣCO_2 system in the marginal EPF) is necessary to explore this possibility. We conclude that introduction and incorporation of respiratory CO_2 into the shell are most likely responsible for the observed depletions in shell $\delta^{13}C$.

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