Marine Biology (2001) 139: 1115–1124
DOI 10.1007/s002270100673

**H. Kennedy · C.A. Richardson · C.M. Duarte
D.P. Kennedy**

Oxygen and carbon stable isotopic profiles of the fan mussel, *Pinna nobilis*, and reconstruction of sea surface temperatures in the Mediterranean

Received: 28 August 2000 / Accepted: 7 July 2001 / Published online: 11 September 2001
© Springer-Verlag 2001

Abstract Stable oxygen and carbon isotope profiles ($\delta^{18}O_{skeletal}$ and $\delta^{13}C_{skeletal}$), taken along the direction of growth from the umbo to the shell margin in shells of the pinnid *Pinna nobilis*, were used to reconstruct sea surface temperatures (SST) in the south-east Mediterranean and ontogenetic records of metabolic CO_2 incorporation. Comparison of the seasonal cycle of SST, predicted from the $\delta^{18}O_{skeletal}$ record of a small (young) rapidly growing pinnid and temperature measured with a continuous in situ recorder showed that *P. nobilis* calcifies under isotopic equilibrium with surrounding seawater, thus indicating that *P. nobilis* shells can be used as a reliable predictor of SST. A 10-year SST record for the south-east Mediterranean was reconstructed from the shell profiles of four pinnid shells of different sizes and ages collected in 1995 and 1996. Reliable resolution of the seasonal SST could only be achieved during the first 4 years of shell growth. As the pinnids grew older, the temperature record was poorly resolved because the shell growth had diminished with age, resulting in time-averaging of the record. The amplitude of the generated seasonal temperature cycle compared favourably (±2°C) with a long-term temperature record from northern Mediterranean waters. Clear seasonal cycles in $\delta^{13}C_{skeletal}$ were observed with an amplitude of ~1.0‰, similar to the calculated seasonal changes in $\delta^{13}C$ of seawater (0.6‰) overlying seagrass meadows. An ontogenetic trend towards less positive $\delta^{13}C_{skeletal}$ values was too large to be attributed to any decrease in $\delta^{13}C$ in seawater resulting from the invasion of anthropogenic CO_2. It is suggested that the temporal changes of $\delta^{13}C_{skeletal}$ are due to incorporation of respiratory CO_2 into the extrapallial fluid and reflect changes in the metabolic activity of the pinnid rather than changes in the isotopic composition of dissolved inorganic carbon within the surrounding seawater.

Communicated by J.P. Thorpe, Port Erin

H. Kennedy (✉) · C.A. Richardson · D.P. Kennedy
School of Ocean Sciences,
University of Wales, Bangor, Menai Bridge,
Ynys Mon, LL59 5EY, UK

E-mail: h.a.kennedy@bangor.ac.uk

C.M. Duarte
Instituto Mediterráneo de Estudios Avanzados,
CSIC-Universitat de les Illes Balears,
C/Miquel Marqués 21, 07190 Esporles,
Balearic Islands, Spain

Introduction

The skeletons of calcareous organisms such as foraminifera, corals and molluscs contain an ontogenetic record of the dynamics of the particular environment they inhabit (Rhoads and Lutz 1980; Andreasson et al. 1999; Khim et al. 2000). The oxygen isotopic record in skeletal structures ($\delta^{18}O_{skeletal}$) of these organisms reflects the temperature and stable isotopic composition of the surrounding seawater at the time of deposition (Urey 1947). Sequential samples taken along the main axis of linear growth have allowed oxygen isotopic profiles, resolved to a subannual level, to be determined thus permitting both growth (Jones et al. 1983; Krantz et al. 1984; Richardson et al. 1999) and temperature reconstructions (Weidman et al. 1994; Buening and Spero 1996; Watanabe and Oba 1999). Carbon isotope ratios from mollusc shells ($\delta^{13}C_{skeletal}$) can record both environmental and/or physiological changes (Krantz et al. 1987; McConnaughey et al. 1997). The $\delta^{13}C_{skeletal}$ records have been used to reconstruct environmental conditions by assuming that carbon isotopes are precipitated in equilibrium with the dissolved inorganic carbon (DIC) of the ambient seawater. Changes in $\delta^{13}C_{skeletal}$ have been used to reconstruct the isotopic composition of DIC ($\delta^{13}C_{DIC}$) and hence cycles of primary production using scallops, *Placopecten magellanaus* (Krantz et al. 1987), upwelling events using *Mytilus edulis* (Killingley and Berger 1979) and stratification of coastal waters using clams, *Spisula solidissima* (Arthur et al. 1983). However, isotopic evidence from studies of other species suggests that physiological factors can also play an important role in defining or modifying $\delta^{13}C_{skeletal}$ (McConnaughay 1989a; Klein et al. 1996). The molluscan shell is precipitated from the extrapallial fluid (EPF) that is contained between the mantle epithelium and inner shell surface. The isotopic composition of the EPF must be indistinguishable from that of seawater when carbon isotopes have been shown to precipitate in isotopic equilibrium with seawater. In some situations, the isotopic composition of the EPF can be modified by a flux of respiratory CO_2 derived from metabolic processes. The presence of respiratory CO_2 in the EPF alters its isotopic characteristics, i.e. it becomes isotopically light relative to seawater. 'Metabolic effects' result when shell is precipitated in isotopic equilibrium with the EPF that has a different (i.e.^{13}C depleted) composition relative to seawater (Tanaka et al. 1986; Klein et al. 1996; McConnaughey et al. 1997). Even a relatively small change in the contribution of metabolic CO_2 to the EPF can result in $\delta^{13}C_{skeletal}$ variations that are of a similar magnitude to the isotopic changes observed in surface ocean water during the seasonal cycle of primary production (Gruber et al. 1999).

Fan mussels, *Pinna nobilis*, are large bivalves indigenous to the Mediterranean where they grow rapidly, up to 1 mm per day, in the first 2 years of life and attain lengths of approximately 65 cm after 12–15 years (Richardson et al. 1999). They occur in sheltered coastal waters between depths of 0.5 to 60 m usually amongst seagrass, *Posidonia oceanica*, meadows and are not generally abundant, although they may be patchily distributed and locally common (Butler et al. 1993). The body of the shell in *Pinna nobilis* is calcitic and unlike many bivalves the surface does not bear any obvious external growth rings. Estimates of age, however, may be obtained by counting the annual growth scars on the inner aragonitic layer that bears the scars of the migration of the posterior adductor muscle. The annual nature of these scars has been validated by determining the seasonal pattern of shell growth using oxygen isotope analysis of sequential samples of shell carbonate and relating the distances of the scars along the muscle scar to the shell (Richardson et al. 1999). Given the rapid rate of shell growth of *P. nobilis* during the first 3–4 years of life, the potential exists for weekly or even daily resolution of seawater temperatures and $\delta^{13}C_{DIC}$. Using fan mussels of different sizes and overlapping ages it should also be possible to construct surface seawater records over decadal periods. In this paper we determine the applicability of $\delta^{18}O_{skeletal}$ and $\delta^{13}C_{skeletal}$ as proxy recorders of temperature and $\delta^{13}C_{DIC}$ respectively and reconstruct the history of environmental variation in the shallow waters of the south-east Spanish Mediterranean. The data are presented by initially calibrating a *P. nobilis*, $\delta^{18}O_{skeletal}$ record with in situ records of seawater temperature change. Subsequently, inferred temperatures, from the $\delta^{18}O_{skeletal}$ variations, in four other specimens of *P. nobilis* are compared with available sea surface temperature (SST) records from the Spanish Mediterranean. Finally, trends in the *P. nobilis* $\delta^{13}C_{skeletal}$ record are discussed.

Materials and methods

Five specimens of *Pinna nobilis* (size range 15–65 cm), part of a group of fan mussels collected by divers in 1995 from three sites (Villaricos, Aguamarga and Rodalquilar) along the south-east Spanish coast (Fig. 1), were studied. At one of these sites, Rodalquilar, a battery-operated temperature recorder (Tinytalk) was tethered to the seabed on 15 September 1995 at a water depth of 3 m amongst *P. nobilis* in a seagrass meadow. Temperature data were recorded at 8-h intervals from its deployment until 24 August 1996. In September 1996, the recorder and a small (15 cm) *P. nobilis* specimen were retrieved from this site. The $\delta^{18}O$ chronology of the *P. nobilis* collected from Rodaquilar in 1996 was

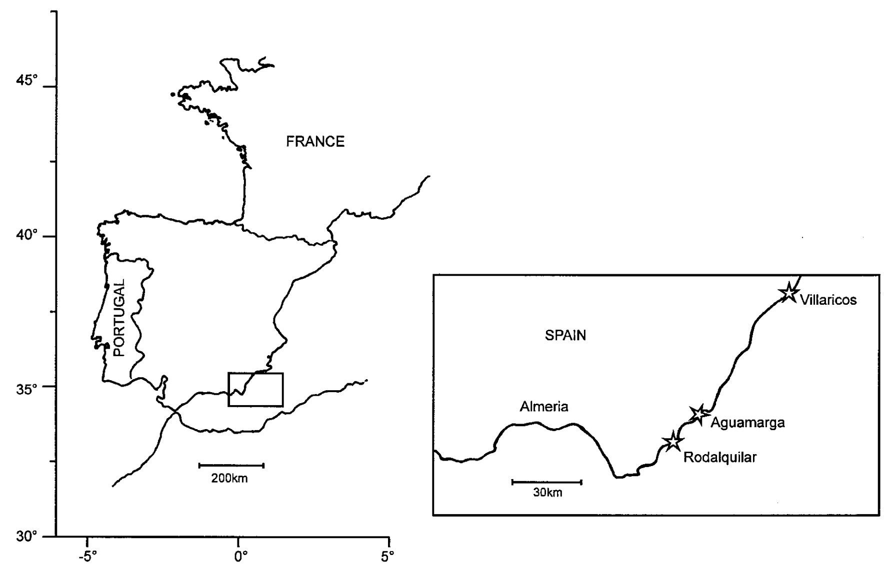

Fig. 1 Location of study area in Spanish Mediterranean (*inset* location of the three sampling sites)

compared with the measured seawater temperature between 1995 and 1996 to assess whether the shell was a reliable recorder of seawater temperature.

The shell growth of *P. nobilis* is rapid during the first 4 years of life but then slows down appreciably (Richardson et al. 1999). Thus it is possible, by choosing appropriate fan mussels of different sizes and ages, to overlap the early periods of shell growth and provide a decadal chronology of the carbon and oxygen isotopic composition of the shell. Four different-sized *P. nobilis* which had been collected in 1995 from Aguamarga and Villaricos and whose age had previously been determined from the adductor muscle scars (Richardson et al. 1999) were analysed. The age of the two fan mussels from Villaricos (V2b, 15 cm and V3a, 25 cm) was estimated at 1 and 2 years old, respectively, whilst those from Aguamarga (Ag1b, 42.1 cm and Ag4a, 53 cm) were 7 and 10 years old respectively. Initially, any encrusting organisms were removed gently by scrubbing the shells with a soft brush. The small (< 15 cm) shells were adorned with conspicuous U-shaped spines over their outer shell surface and these were removed sequentially along the main axis of shell growth. The spines on the larger (older) shells had been worn away and for these shells discrete samples were drilled sequentially along the main axis of shell growth at 0.25- to 0.5-mm intervals using a 0.6-mm-diameter dental drill. An examination of the shell showed that the spines are an extension of the outer layer of the shell and the prismatic structure is laid down in both the spine and the outer shell layer contiguously. The depth of drilling was carefully controlled so that no aragonite from the inner muscle scar could contaminate the calcite sample. The calcite of spines (when present) or drilled samples (when spines had been worn away) was homogenised separately. These samples were pre-treated to remove organic material in a low-temperature oxygen plasma for 4.5 h (Swart 1981) and reacted in vacuo with > 100% H_3PO_4 at 25°C. Evolved CO_2 was cryogenically cleaned and the isotopic composition measured.

Replicate 1-l seawater samples, collected for isotopic analysis, were obtained in September 1996 from Aguamarga and Rodaquilar using a Niskin water bottle. A 100-ml subsample was taken in a glass bottle, sealed and stored for $\delta^{18}O$ analysis. Replicate 100-ml seawater samples for $\delta^{13}C_{\mathrm{seawater}}$ were retained in ground glass bottles and immediately poisoned with $HgCl_2$. On return to shore, subsamples (10 ml) were removed and filtered through a 0.4-µm Nuclepore filter into a glass ampoule, flushed with nitrogen and sealed. In the laboratory, the isotopic composition of the water ($\delta^{18}O_{\mathrm{seawater}}$) was determined using the conventional CO_2–H_2O equilibration method (Epstein and Mayeda 1953). The $\delta^{13}C_{\mathrm{DIC}}$ samples were cracked under vacuum, acidified and stripped with N_2. The CO_2 evolved was cryogenically separated from water and other gases using a modification of the method of (Kroopnick 1974).

Temperature was calculated from the isotopic ratios of the pinnids calcite using the palaeotemperature equation of O'Neil et al. (1969): $T\,°\mathrm{C} = 16.9–4.38(\delta_c–\delta_w) + 0.01(\delta_c–\delta_w)^2$, where: δ_c is the $\delta^{18}O$ value of shell calcite (reported VPDB) and δ_w is equivalent to $\delta^{18}O_{\mathrm{seawater}}$ (reported VSMOW) –0.27‰.

Additional isotopic measurements were made on the *P. nobilis* flesh and samples of *P. oceanica* collected from Aguamarga in 1996. The flesh was removed from the pinnids and frozen. The youngest leaves of the seagrass, which had no apparent epiphyte cover, were rubbed clean with a dry filter paper and also stored frozen. Both sample types were subsequently dried and the material homogenised. For isotopic analysis of the organic carbon ($\delta^{13}C_{\mathrm{POC}}$), subsamples were loaded into precombusted silver boats (500°C, 3 h), acidified with HCl (10%) and dried. Copper and precombusted copper oxide (910°C, 3 h) was added to the boats which were then loaded into precombusted quartz tubes and sealed under vacuum. These tubes were combusted at 910°C for 3 h and allowed to cool slowly to room temperature. CO_2 was cryogenically separated from the resulting gases and its isotopic composition measured.

All isotopic measurements were determined using a VG or PDZ-EUROPA mass spectrometer. The results are reported in per mil (‰) deviations relative to VPDB or VSMOW. The overall analytical precision for $\delta^{18}O$ and $\delta^{13}C$ of calcite samples are 0.11‰ and 0.04‰ respectively; for $\delta^{18}O_{H2O}$ and $\delta^{13}C_{\mathrm{DIC}}$ are 0.06 and 0.07‰ respectively; and for $\delta^{13}C_{\mathrm{POC}}$ is 0.08‰ based on analyses of internal laboratory standards run concurrently with all the samples.

Results

Validation of *Pinna nobilis* as a temperature recorder

The measured SST record from Rodalquilar (Fig. 2a) showed an initial sharp increase after deployment, reaching a maximum of ~22°C in late September/early October 1995. SST subsequently decreased through late October to December and reached minimum temperatures between January and March (14–15°C). Early warming of the water occurred during April/May and the temperature increased and stabilised during August with a mean temperature of 25.2 ± 0.9°C. The seasonal warming and cooling of the seawater at this coastal site does not follow a smooth transition but is punctuated by periods of rapid cooling. These fluctuations may have arisen as a result of the intrusion of cold water into the south-west facing bay due to the influence of strong offshore winds which can result in localised and temporally variable upwelling.

The oxygen isotopic composition of the seawater, collected in September 1996, was 1.04‰, 2 SD = 0.03

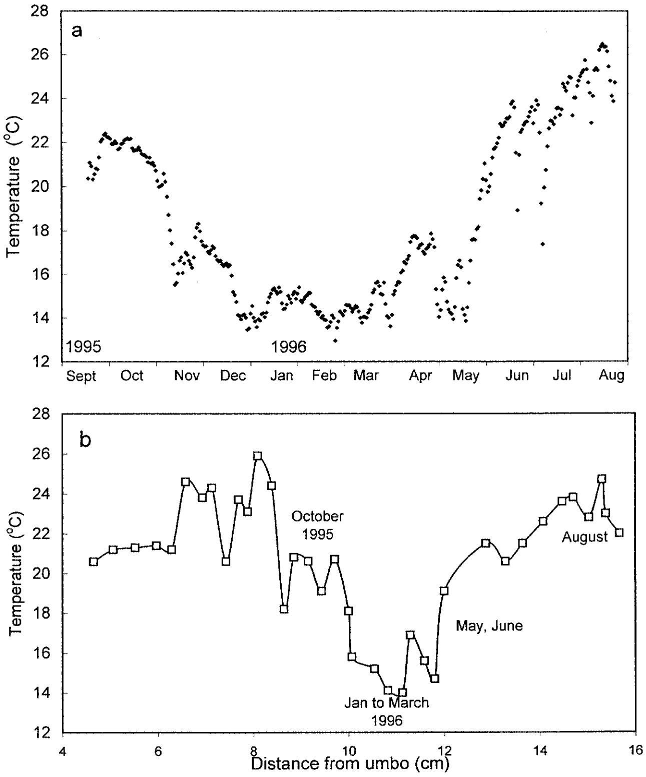

Fig. 2a, b Seasonal variation in the seawater temperatures: **a** measured in situ, using a temperature logger, and **b** predicted from the $\delta^{18}O_{\mathrm{skeletal}}$ recorded in the spines of a *Pinna nobilis* from Rodalquilar and is close to the value of 1.13‰ reported by Epstein and Mayeda (1953) for Mediterranean water of salinity 38.8 at 50 m depth. In the palaeotemperature calculation we assume that the δ^{18}O (salinity) of the water remains unchanged. There is little or no precipitation in this region of the Mediterranean and any changes in salinity are more likely to be due to evaporation. Monthly averaged data (Pico 1990) support this view with an annual salinity for this region of 36.85, 2 SD = 0.46. Variation in salinity of this magnitude is equivalent to approximately 0.14‰, about our measured δ^{18}O seawater value.

The U-shaped spines present from the umbo to the shell margin in the pinnid removed from Rodalquilar were sequentially sampled to provide the isotopically derived temperature estimates (Fig. 2b). The earliest sampled spine at 4.25 cm from the umbo predicts a temperature of $\sim$20°C and subsequently rises to $\sim$25°C between 6 and 8 cm from the umbo. We infer that this period of spine production preceded the period of deployment of the in situ temperature sensor (which only recorded an early maximum of $\sim$22°C) and therefore only the spine profile after 8 cm is used for comparison. From this point the derived $\delta^{18}O_{\mathrm{skeletal}}$ temperature data show three distinct periods that can be directly compared with the recorded in situ temperature. The shell increased in length about 2 cm ($\sim$8–10 cm) between October and January 1995, followed by a slower period of growth with an increase of $\sim$1 cm (10–11 cm) between January and March 1996. A period of rapid growth occurred between May and 25 August 1996 when the seawater temperature increased from 14°C to 24°C and shell length increased from 12 cm to 16 cm. The temperature record predicted from $\delta^{18}O_{\mathrm{skeletal}}$ of the spines closely agreed with the in situ measured temperatures and provided a reliable indication that the calcite of the spines on *P. nobilis* was deposited in oxygen isotopic equilibrium with seawater. In this shell, the sampling resolution using the U-shaped spines (0.16–0.87 cm apart) was insufficiently detailed to record the period of slow winter growth or the large amplitude changes of short duration in the seawater temperature.

The potential of the fan mussel shell for reconstruction of decadal temperature trends

Long-term SST records, with which to compare our data from large pinnid shells, are sparse in the Mediterranean. The nearest locations where comparable records exist (daily/weekly) are the Bay of Blanes (1992–1996) and L'Estartit (1985–1993) in the north-west Mediterranean (Pascual and Flos 1984; Duarte et al. 1999). The decadal temperature record from this region shows seasonal changes in seawater temperatures of about 12–13°C (Fig. 3); coldest temperatures (11–13°C) occur between January and March whilst maximum temperatures (23–27°C) occur in August/September. Although these measurements were collected in the north-western Mediterranean, distant from the study location at Rodalquilar, comparison of the seasonal temperature changes measured in 1995 and 1996 at Blanes and Rodaquilar shows close agreement (Fig. 4). The rate of spring warming of the seawater is essentially the same at both sites; there are similar maximum summer SSTs, but during the autumn and winter the seawater is warmer by $\sim$2°C at Rodalquilar relative to the Bay of Blanes. The seasonal variation in the average monthly SSTs for these regions (Fig. 4; data from Pico 1990) shows that the longer term trends are also similar at these general locations. The comparability of the SST data suggests that it would be feasible to compare the $\delta^{18}O_{\mathrm{skeletal}}$ temperature chronologies derived from the fan mussels in the south-east with the measured SST data from the north-east Spanish Mediterranean.

Temperature records reconstructed from shell profiles of four selected pinnids were characterised by successive seasonal temperature cycles (Fig. 5). The records at Aguamarga were derived from discrete samples drilled sequentially along the main axis of shell growth, while those from Villaricos were derived from the spines that were still present along the outer shell surface. Maximum amplitudes in the temperature records reconstructed from the larger pinnids from Aguamarga were

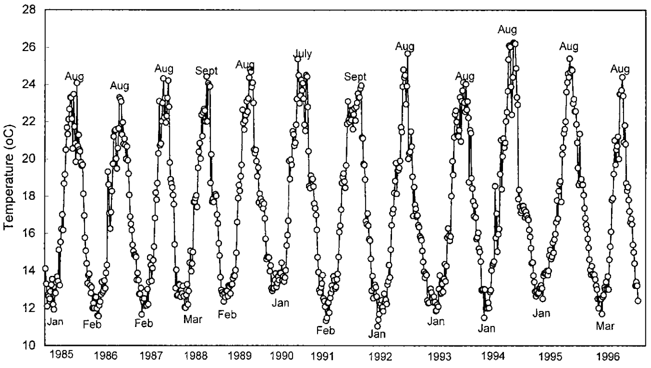

Fig. 3 A decadal seasonal variation in the seawater temperature of the north-east Spanish Mediterranean between 1985 and 1996 compiled from daily/weekly records collected from L'Estartit and Bay of Blanes

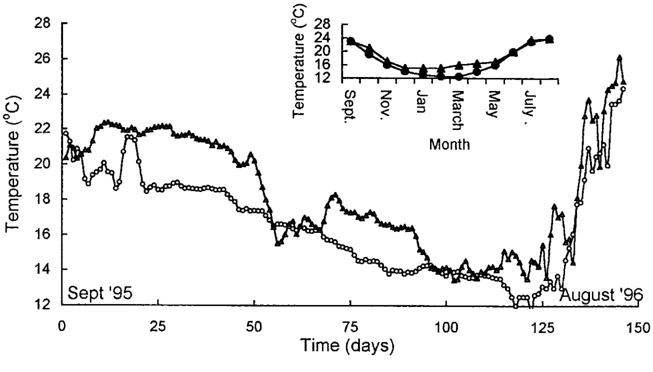

Fig. 4 Comparison between the seawater temperature measured at the study site, Rodalquilar (Δ) and the Bay of Blanes (○), north-west Mediterranean 1995 and 1996. *Inset* Seasonal variation in the monthly surface seawater temperature from the general location of the study site (▲) and the Bay of Blanes (●). Data from Pico (1990)

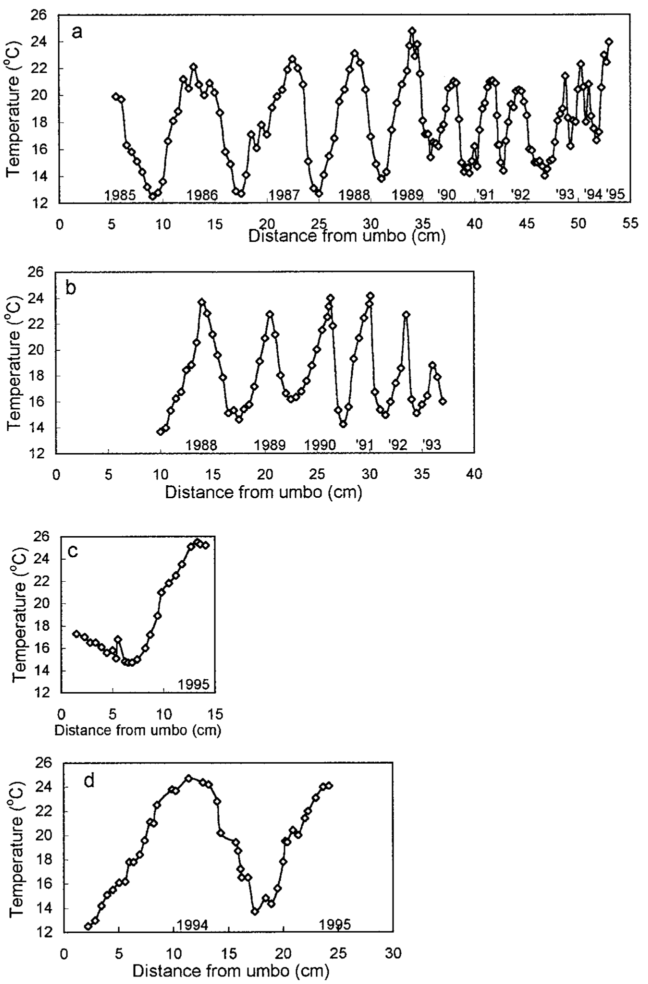

Fig. 5a–d Temperature, predicted from $\delta^{18}O_{skeletal}$, versus distance from the umbo: for drilled shells from Aguamarga (**a** Ag4a and **b** Ag1b) and spines on shells from Villaricos (**c** V2b and **d** V3a)

observed during the first 4 years of growth (Fig. 5a, b). The amplitude decreased appreciably as the pinnid increased further in size and became older. Within a shell, the distance between successive annual maxima provides information on the annual growth rate of the mollusc, and this distance also decreased with increasing age of the mollusc. Since the age of each pinnid could be determined from the posterior adductor muscle scar rings, the year appropriate to each of the temperature maxima and minima was assigned. The $\delta^{18}O$ chronology from the largest Aguamarga specimen (Ag4a) shows that shell was secreted over a 10-year period between 1985 and 1995 and a good resolution of annual cycles was recorded between the latter half of 1985 and 1989 (Fig. 5a). It can be inferred, from the relative amplitude of the predicted temperature record, that the slow growth rate of the Aguamarga (Ag4a) pinnid over the last few years of its life resulted in a smoothing of the temperature record and hence to an underestimation of the actual temperature range. Samples drilled from a smaller Aguamarga shell (Ag1b) record changes between 1988 and 1993 (Fig. 5b). The first 4 years of growth (1988–1991) in this pinnid show good resolution of annual temperature changes that overlap with the record from the larger Aguamarga pinnid (Ag4a). The spines from the two smallest shells, from Villaricos, provide chronologies for 1994 and 1995 (Fig. 5c, d) and have a faster shell growth rate than the two shells from Aguamarga. The reconstructed temperature records between the Aguamarga pinnids and the smaller Villaricos fan mussels overlap.

Calendar dates were assigned to all of the samples that had been analysed (Klein et al. 1996) by matching the temperature estimates derived from the $\delta^{18}O_{skeletal}$ record, with the daily/weekly measurements of SST from the north-west Mediterranean. As *P. nobilis* aged, an increasing part of the measured seasonal temperature was not recorded in $\delta^{18}O_{skeletal}$. Shell growth must have ceased or slowed down so much that neither sampling strategy could resolve the temperature record for a period during both the coldest winter and the warmest summer temperatures (Table 1). For example, in the Aguamarga shell (Ag4a) between 1986 and 1988, growth cessation in the winter was for a period of 80–94 days, from late December/early January to March. As the pinnid aged, the period when no growth occurred widened and from 1990 onwards shell deposition ceased for a period of ~170 days (5 months) from December to May.

Table 1 The period where no growth occurred, identified by calendar date (0–365), and its length (in days) for which no temperature change (°C) was recorded in the shell of the large pinnid from Aguamarga (Ag4a). The months relating to equivalent calendar dates are: May (121–151); July (182–212); August (213–243); September (244–273); and October (274–304). The minimum and maximum temperatures recorded in each year are also reported

Minimum				Maximum		
Year	Period	Length	Temperature	Period	Length	Temperature
1986	3–83	80	12.5	232–256	24	22.1
1987	5–92	87	12.7	212–268	56	22.7
1988	349–78	94	12.7	180–230	50	23.1
1989	351–126	140	13.8	231–252	21	23.8
1990	326–135	174	15.4	177–279	102	21.0
1991	341–147	171	14.3	192–277	85	21.1
1992	329–115	151	14.4	193–250	57	20.4
1993	344–124	145	14.0	169–279	110	21.4
1994	304–166	227	18.0	191–263	72	20.6

A similar, but less extreme, situation occurred when water temperature was warmest. In the first 3 years the records from only a small part of July/August have not been resolved, but again by 1990 shell extension was not fast enough to record any temperature changes between July and October (calendar dates 182–304; Table 1). At this time the pinnids were only actively growing 2–4 months of the year. The cumulative effect of these features was that after the first few years of growth, the maximum temperature recorded in any of the shells was lower than the maximum measured SST (Fig. 6a). The seasonal minima showed a similar pattern in that the minimum temperature recorded in the shell increased with the age of the pinnid. The minima in SST in the north-western Mediterranean should be ~2°C colder than along the south-east Spanish coast (Fig. 4); however, the shell-derived temperatures were not consistently 2°C warmer, but were ± 2°C of the expected minimum temperature throughout the pinnids' life (Fig. 6b).

The $\delta^{13}C_{skeletal}$ profiles show cyclic variations with a trend towards lighter values with increasing age of the pinnid (Fig. 7). The data could reflect either equilibrium or kinetic precipitation. Kinetic effects are not implicated because there were no significant co-variations between the $\delta^{13}C_{skeletal}$ and $\delta^{18}O_{skeletal}$ values (McConnaughy 1989b). Using the calendar dates assigned to the reconstructed $\delta^{18}O_{skeletal}$ temperature records, $\delta^{13}C_{skeletal}$ can be presented as variation with time rather than distance from the umbo (Fig. 8). The trend towards lighter values of $\delta^{13}C_{skeletal}$ remains fairly constant with time and regression analysis of sequential maxima, minima or the whole data set with time results in very similar estimates of the gradient (0.00051, 2 SD = 0.00007‰ day^{-1} or ~0.18‰ year^{-1} respectively). The majority of the maximum $\delta^{13}C_{skeletal}$ values are recorded between days 121 and 151, i.e. during May (Table 2). The timing of the minimum is more variable. In shell Ag4a they are recorded from July to the end of October (days 182–304), while in Ag1b and V3a they are always recorded in August (days 213–243). The amplitude of the annual cycle in $\delta^{13}C_{skeletal}$ remains fairly constant with time in Aguamarga pinnids (Ag4a and Ag1b): average = 0.59‰, 1 SD = 0.08‰, $n = 6$ and average = 0.87‰, 1 SD = 0.17‰, $n = 5$, respectively.

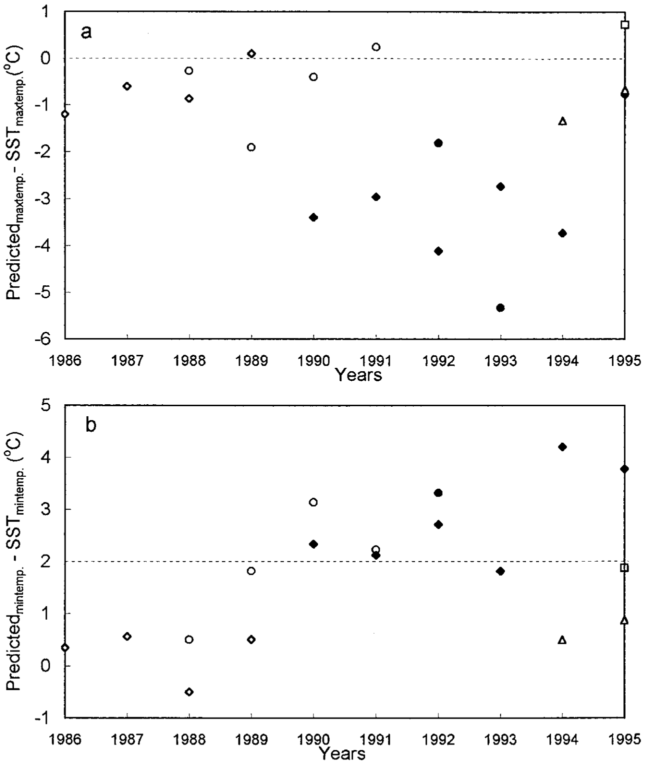

Fig. 6 Deviation of **a** maximum and **b** minimum temperatures predicted using $\delta^{18}O_{skeletal}$ from the drilled shells from Aguamarga, Ag4a(◇) and Ag1b(○), from the spines on shells from Villaricos, V2b(❑) and V3a(Δ), and the SST measured in the north-west Mediterranean. *Open symbols* display data from first year(s) of growth for comparison with later year(s) growth (*filled symbols*)

The assumption that $\delta^{13}C_{skeletal}$ represents the change in $\delta^{13}C_{DIC}$ due to seasonal variations in primary production can be tested by comparing the amplitude of the seasonal cycle in $\delta^{13}C_{skeletal}$ with that of $\delta^{13}C_{DIC}$.

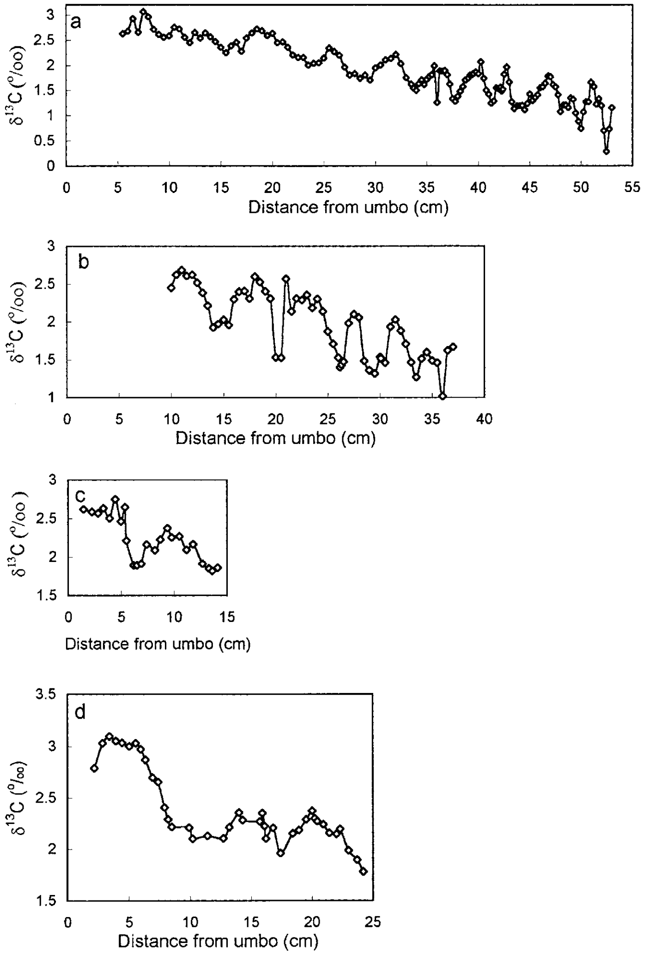

Fig. 7a–d $\delta^{13}C_{skeletal}$ versus distance from the umbo (cm): for drilled shells from Aguamarga (**a** Ag4a and **b** Ag1b) and for spines on shells from Villaricos (**c** V2b and **d** V3a)

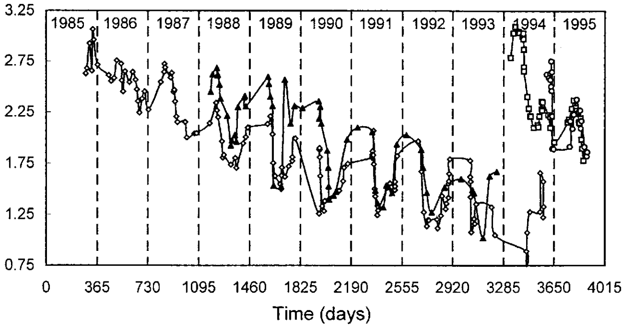

Fig. 8 $\delta^{13}C_{skeletal}$ versus time, determined from assigned calendar dates: for drilled shells from Aguamarga (◇ Ag4a and ▲ Ag1b) and for spines on shells from Villaricos (❑ V2b and ○ V3a). Temporal trends describing rate of change of $\delta^{13}C_{skeletal}$(‰) with time (years) are: Ag4a, $y = -0.0005x + 2.7$ $r^2 = 0.78$; Ag1b, $y = 0.0005x + 3.0$ $r^2 = 0.55$

Table 2 Timing, identified by calendar date (0–365), when the most positive (maximum) and negative (minimum) skeletal $\delta^{13}C$ values have been observed. The months relating to equivalent calendar dates are given in the legend to Table 1

	Maximum			Minimum		
Year	Ag4a	Ag1b	V3a	Ag4a	Ag1b	V3a
1986	140			305		
1987	157			279		
1988	124	130		263	230	
1989	162	130		225	225	
1990	135	131		171	237	
1991	151	149		186	228	
1992		29			213	
1993						
1994			89			221
1995			147			

Unfortunately the possible extent of seasonal variations in $\delta^{13}C_{DIC}$ at the study sites is not known. The value of $\delta^{13}C_{DIC}$ was only measured during the fieldwork in September 1996 and is 1.38‰ (1 SD = 0.23‰, $n = 14$). It is likely that community production and respiration mediate the major isotopic changes in the ambient water. In the oligotrophic waters of the littoral Spanish Mediterranean, phytoplankton production is not strongly seasonal (Duarte et al. 1999) and production is estimated to be ~130 g C m^{-2} year^{-1} (Agusti and Duarte 2000). Average production of *Posidonia oceanica* beds is ~350 g C m^{-2} day^{-1} (Duarte and Chiscano 1999) and strongly seasonal. Hence at our study site it is more likely that the growth pattern of the seagrass rather than phytoplankton that is the main modulator of $\delta^{13}C_{DIC}$ at annual time scales.

An estimate of the annual change in $\delta^{13}C_{DIC}$ can be made from a simple mass balance equation: $[DIC_{Winter}]\,\delta^{13}C_{Winter} = [DIC_{Summer}] \times \delta^{13}C_{Summer} + \Delta DIC \times \delta^{13}C_{Seagrass}$, where $[DIC_{Winter}] = 2{,}000\ \mu M$, $\delta^{13}C_{Winter} = 1.0$, $\Delta DIC = 120\ \mu M$ (Frankignoulle and Disteche 1984) and $\delta^{13}C_{Seagrass} = -12.2$‰ (this study). We have assumed that advection and air sea exchange were insignificant and used our measured values of the isotopic composition of the seagrass and annual DIC concentration changes measured within seagrass meadows elsewhere in the Mediterranean (Bay of Calvi, Corsica; Frankignoulle and Disteche 1984). The $\delta^{13}C_{Summer}$ was calculated to be 1.6‰ and so the seasonal change in $\delta^{13}C_{DIC}$ is estimated at 0.6‰, close to the observed amplitude (0.6 and 0.9‰) of the cyclic variations in $\delta^{13}C_{skeletal}$ (Fig. 7).

Alternatively, the shell $\delta^{13}C_{skeletal}$ profile could be recording changes in the $\delta^{13}C_{DIC}$ of the EPF, an internal DIC reservoir from which the shell precipitates. The isotopic composition of the EPF can be modified by a variable flux of respiratory (^{13}C depleted) CO_2 (McConnaughey 1989a). In which case, the seasonal change in $\delta^{13}C_{skeletal}$ must be taken to represent seasonal variation in the animal's metabolic activity. An isotopic mixing equation has been used to determine the relative proportion of respired carbon that would have to be incorporated into the precipitating carbonate to yield the observed seasonal cycle (McConnaughey et al. 1997): $R \times (\delta^{13}C_{resp}) + (1-R) \times (\delta^{13}C_{seawater}) = \delta^{13}C_{shell} - \epsilon_{Cl\text{-}HCO3^-}$, where R is the fraction of respired carbon in the shell, $\delta^{13}C_{resp}$ and $\delta^{13}C_{seawater}$ are the isotopic compositions of HCO_3^- derived from respiration and seawater HCO_3^- respectively and $\epsilon_{Cl\text{-}HCO3^-}$ is the calcite-bicarbonate equilibrium fractionation factor.

The isotopic composition of respired organic matter depends on the isotopic composition of the organism's tissue. The average $\delta^{13}C$ of the tissue removed from three of the pinnids was –18.3 ‰ (1 SD = 0.64‰, $n = 3$) and $\delta^{13}C_{resp}$ is taken to be 0.5‰ heavier than the organic material being respired (McConnaughey et al. 1997). These values can be used to estimate the increased percentage of respired carbon (3% and 4.5%) that would cause the observed amplitude (0.6‰ and 0.9‰) in the seasonal cycle of $\delta^{13}C_{skeletal}$ in the larger (Ag4a) and smaller (Ag1b) pinnid respectively. The steadily decreasing background of the $\delta^{13}C_{skeletal}$ profiles may also either record changes in the $\delta^{13}C_{DIC}$ of the seawater or the EPF.

Discussion

The temperature variation measured in situ at Rodalquilar does not follow the seasonal cycle characteristic of coastal sites seen at another location in the Spanish Mediterranean (Duarte et al. 1999), but experiences rapid, 1- to 2-week deviations in SST. Nevertheless, the shell recovered from Rodalquilar and subsequently analysed provides evidence that equilibrium deposition of $\delta^{18}O$ occurs and *Pinna nobilis* can be used as a reliable predictor of SST providing the $\delta^{18}O$ of the ambient water is also known. The predicted seawater temperature records, from four further shells from two different sites, display a progressive decline in amplitude of the seasonal temperature cycle with increasing age of the *P. nobilis* (Fig. 5). In their 4th or 5th year the predicted summer maximum seawater temperatures are more than

~2°C colder than the measured SST (Fig. 6a). The reduction in amplitude of the seasonal changes probably reflects a decreasing radial growth rate of the shell with age and season. There is cessation or very slow shell growth when the water temperatures are at their warmest and coldest. In the first few years pinnid Ag4a actively precipitated shell for 8–11 months of the year, but later on in its life, the period was reduced to 3–6 months. There appears to be no minimum or maximum threshold temperature for shell deposition but a gradual focussing of growth into a shorter period of time as the mollusc ages. Given the distance between drilled samples was 0.5 cm and then 0.25 cm in the larger pinnid from Aguamarga (Ag4a), the integration time of each sample varies between 2 weeks and 5 months. With access to a community of *P. nobilis* having a greater range of sizes than collected in this study, and/or access to museum specimens, it may be possible to reconstruct longer chronologies of temperature by using overlapping temperature records derived from the first few years of growth of these shells.

No kinetic effects were observed as the $\delta^{18}O$ and $\delta^{13}C$ values of the precipitated shell were found to vary independently, i.e. there was no significant correlation between $\delta^{18}O_{skeletal}$ and $\delta^{13}C_{skeletal}$ values. What is not known is whether precipitation occurred in carbon isotopic equilibrium with an EPF that is indistinguishable from that of seawater or with an EPF that has been modified by metabolic effects. Thus the cyclic variation in $\delta^{13}C_{skeletal}$ could either reflect seasonal changes in the ambient $\delta^{13}C_{DIC}$ or seasonal changes in the metabolic activity of the pinnid.

The general co-incidences between the most positive and negative $\delta^{13}C_{skeletal}$ values, the timing of the respective maximum and minimum in net primary production in seagrass meadows (Marba et al. 1966), and the magnitude of the annual calculated and predicted change in $\delta^{13}C_{DIC}$ all suggest that the annual variation in $\delta^{13}C_{skeletal}$ reflects the seasonal changes in the seawater $\delta^{13}C_{DIC}$.

While the seasonal cycle in $\delta^{13}C_{skeletal}$ is consistent with the modulation of seawater $\delta^{13}C_{DIC}$ by net primary production, the trend towards lighter values with increasing age of the pinnid (Figs. 7, 8) cannot be so easily reconciled. One possible mechanism that could explain the trend is that the decrease in $\delta^{13}C_{DIC}$ value is due to the uptake of atmospheric CO_2 derived from fossil fuel combustion (Quay et al. 1992; Bacastow et al. 1996; Gruber et al. 1999). This anthropogenic perturbation of the ocean's ^{13}C reservoir, the Suess effect, has been determined from direct measurements of inter-annual changes in $\delta^{13}C$ of SST in the Pacific, Indian and Atlantic Oceans (Quay et al. 1992; Bacastow et al. 1996; Gruber et al. 1999). It has also been inferred from isotopic records in sclerosponges (Druffel and Benavides 1986) and could also be recorded in other long-lived carbonate-secreting organisms. These studies have reported a range for the rate of change in $\delta^{13}C_{DIC}$ of –0.007‰ to –0.025‰ year^{-1}, far smaller than our measured decrease in $\delta^{13}C_{skeletal}$ of ~0.18‰ year^{-1}. This suggests that it is unlikely that $\delta^{13}C_{skeletal}$ represents the gradual decrease in $\delta^{13}C_{DIC}$ due to influx of anthropogenic CO_2 into Mediterranean surface waters and metabolic effects are implicated.

Our measurements of the seawater $\delta^{13}C_{DIC}$ at the study sites also provide evidence to suggest that the pinnid shell does not record the variation of $\delta^{13}C_{DIC}$ in the ambient seawater. Although the $\delta^{13}C_{DIC}$ was only measured in September 1996, we can use it to compare calculated equilibrium and measured values of $\delta^{13}C_{skeletal}$ value at the margin of the shell (i.e. the shell deposited just prior to collection). Since ~90% of DIC is in the form of bicarbonate, we have used this value and the calcite-bicarbonate equilibrium fractionation factor, $\epsilon_{\text{Cl-HCO3}^-} = \sim 1$‰ (Romanek et al. 1992), to calculate the equilibrium value for $\delta^{13}C_{skeletal}$ as 2.38‰. The values measured at the margin of the pinnid shells 1.16‰, 1.78‰ and 1.86‰ for Ag4a, V3a and V2b shells, respectively, are all lighter than the calculated equilibrium value. The degree of isotopic disequilibrium, at the margin of the shell, was observed to increase with the age of the pinnid. In the large 10-year-old pinnid from Aguamarga (Ag4a), the difference between calculated equilibrium and measured $\delta^{13}C_{skeletal}$ was 1.36‰, whereas for the younger pinnids from Villaricos, V3and V2b, age 1.5 years and 9 months, the differences were 0.64‰ and 0.56‰ respectively. In contrast, the difference between the calculated equilibrium and $\delta^{13}C_{skeletal}$ values measured during September of the first year of growth was much smaller, +0.26 and –0.25‰ for Ag4a and V3a respectively. This suggests that the long-term trend in decreasing $\delta^{13}C_{skeletal}$ values is more probably due to an increase in the incorporation of metabolic CO_2. Taking the $\delta^{13}C_{skeletal}$ values estimated to have been deposited each September in pinnid Ag4a (2.64, 2.15, 1.80, 1.62, 1.48‰), the contribution of respired carbon incorporated into the precipitating shell over these 5 years increases from ~0 to 1.4, 3.2, 4.2 and finally 4.9%. At the margin of the shells, Ag4a, V3a and V2b, the fraction of shell carbonate-carbon derived from metabolic CO_2 is estimated to be 6.4, 3.1 and 2.7% respectively. These values are consistent with other studies where the proportion of respired carbon dioxide incorporated in the shells is usually small, 0–25% (Paull et al. 1989; Klein et al. 1996; McConnaughey et al. 1997). These trends have been generally interpreted as reflecting physiological change (Jones et al. 1986 ; Krantz et al. 1987). In juvenile molluscs, metabolic energy is used to support fast growth, but in adults, metabolic energy is used to support gametogenesis and shell growth is subsequently reduced. Maturation and the associated slower growth in the adult stage can be accompanied by a change to increasingly lighter $\delta^{13}C_{skeletal}$ values (Romaneck and Grossman 1987). *P. nobilis* reaches sexual maturity when it is 1–2 years old (de Gaulejac 1995); however, no obvious change in the trend of $\delta^{13}C_{skeletal}$ towards lighter values could be discerned at this time.

Metabolic effects could also explain the seasonal cycles in $\delta^{13}C_{skeletal}$. Variations in the animal's metabolic activity is related to both temperature and food availability. As these are also seasonally variable, the proportion of metabolic CO_2 that is incorporated during shell secretion will also change and a seasonal variation in $\delta^{13}C_{skeletal}$ would not be unexpected. Only a small increase (3–4.5%) of respired carbon is necessary to effect such a change. However, the amplitude of the seasonal cycle in $\delta^{13}C_{skeletal}$ does not vary much with age. If growth, size and metabolic rate can all influence the extent to which respiratory CO_2 is incorporated into the EPF, then it might be expected that this should also influence the magnitude of the observed seasonal changes in $\delta^{13}C_{skeletal}$.

The results presented show that the shells of *P. nobilis* provide reliable witnesses to the change in temperature of the ambient Mediterranean waters. Because of the very rapid growth rates of young *P. nobilis* shells, the extracted temperature records can provide high resolution (biweekly averages). The capacity of *P. nobilis* to be used as a recorder of temperature change may potentially help resolve the paucity of interannual seawater temperature records in the Mediterranean. In contrast, the carbon isotopic composition of the shell appears to be affected by ontogenetic changes in metabolism and growth, confounding possible expected trends derived from seasonal cycles of community production or invasion of anthropogenic CO_2 across the air–sea interface. The use of *P. nobilis* shells as temperature loggers, however, confirms the potential of long-lived organisms as reliable recorders of environmental change in the marine system.

Acknowledgements We would like to thank L. Hooper for her help during 1995 and S. Proud for her help during both the 1995 and 1996 fieldwork program. The project was funded with assistance from the joint British Council–Spanish Ministry of Education Programme Acciones Integradas (Ref 50B)and project CL 195–1805 of the Spanish Interministerial Commission of Science and Technology (CICYT). A part of this work was undertaken using support provided to H.K. by NERC JREI (GR3/EOO68) and the Ministerio de Education y Culture, Spain. We would also like to thank the authorities of the Parque Natural del Cabo de Gata-Nijar for permission to work in the park's waters.

References

Agustí S, Duarte CM (2000) Strong seasonality in phytoplankton cell lysis in the NW Mediterranean littoral. Limnol Ocean 45:940–947

Andreasson FP, Schmitz B, Jonsson E (1999) Surface water seasonality from stable isotope profiles of *Littorina littorea* shells: implication for palaeoenvironmental reconstruction of coastal areas. Palaios 14:273–281

Arthur MA, Williams DF, Jones DS (1983) Seasonal temperature-salinity changes and thermocline development in the mid-Atlantic Bight as recorded by the isotopic composition of bivalves. Geology 11:655–659

Bacastow RB, Keeling CD, Lueker TJ, Wahlen M, Mook WG (1996) The ^{13}C Suess effect in the world surface oceans and its implications for oceanic uptake of CO2: analysis of observations at Bermuda. Global Biogeochem Cycles 10:335–346

Buening N, Spero HJ (1996) Oxygen and carbon isotope analyses of the articulate brachiopod *Laqueus californianus*: a recorder of environmental changes in the subeuphotic zone. Mar Biol 127:105–114

Butler AJ, Vincente N, Gaulejac B de (1993) Ecology of the pterioid bivalves *Pinna bicolor* Gmelin and *Pinna nobilis* L. Mar Life 3:37–45

Druffel ERM, Benavides LM (1986) Input of excess CO_2 to the surface ocean based on $^{13}C/^{12}C$ ratios in a banded Jamaican sclerosponge. Nature 321:58–61

Duarte CM, Chiscano CL (1999) Seagrass biomass and production: a reassessment. Aquat Bot 65:159–174

Duarte CM, Agustí S, Kennedy H, Vaqué D (1999) The Mediterranean climate as a template for Mediterranean marine ecosystems: the example of the north-east Spanish littoral. Prog Oceanogr 44:245–270

Epstein S, Mayeda T (1953) Variations of ^{18}O content of waters from natural sources. Geochim Cosmochim Acta 4:213–224

Frankignoulle M, Disteche A (1984) CO_2 chemistry in the water column above a *Posidonia* seagrass bed and related air-sea exchanges. Oceanol Acta 7:209–219

Gaulejac B de (1995) Mise en évidence de l'hemaphrodisme successif à maturation asynchrone de *Pinna nobilis* (L.) (Bivalvia: Pterioidea). C R Acad Sci Ser 3 318:99–103

Gruber N, Keeling CD, Bacastow RB, Guenther PR, Lueker TJ, Wahlen M, Meijer HA, Mook WG, Stocker TF (1999) Spatiotemporal patterns of carbon-13 in the global surface oceans and the oceanic Suess effect. Global Biogeochem Cycles 13:307–335

Jones DS, Williams DF, Arthur MA (1983) Growth history and ecology of the Atlantic surf clam *Spisula solidissima* (Dillwyn) as revealed by stable isotopes and annual shell increments. J Exp Mar Biol Ecol 73:225–242

Jones DS, Williams DF, Romanek CS (1986) Life history of symbiont-bearing giant clams from stable isotope profiles. Science 231:46–48

Khim BK, Woo KS, Je JG (2000) Stable isotope profiles of bivalve shells: seasonal temperature variations, latitudinal temperature gradients and biological carbon cycling along the east coast of Korea. Cont Shelf Res 20:843–861

Killingley JS, Berger WH (1979) Stable isotopes in a mollusc shell: detection of upwelling events. Science 205:186–188

Klein RT, Lohmann KC, Thayer CW (1996) Sr/Ca and $^{13}C/^{12}C$ ratios in skeletal calcite of *Mytilus trossulus*. Covariation with metabolic rate, salinity and carbon isotopic composition of seawater. Geochim Cosmochim Acta 60: 4207–4221

Krantz DE, Jones DS, Williams DF (1984) Growth rates of the sea scallop, *Plactopecten megellanicus*, determined from the $^{18}O/^{16}O$ record in shell calcite. Biol Bull (Woods Hole) 167:186–199

Krantz DE, Williams DF, Jones DS (1987) Ecological and palaeological information using stable isotope profiles from living and fossil molluscs. Palaeogeogr Palaeoclimatol Palaeoecol 58:249–266

Kroopnick P (1974) Correlations between ^{13}C and CO_2 in surface waters and atmospheric CO_2. Earth Planet Sci Lett 22:397–403

Marbà N, Cebrián J, Enríquez S, Duarte CM (1966) Growth patterns of Western Mediterranean seagrasses: species-specific responses to seasonal forcing. Mar Ecol Prog Ser 133:203–215

McConnaughey TA (1989a) ^{13}C and ^{18}O disequilibrium in biological carbonates, I, Patterns. Geochim Cosmochim Acta 53:151–162

McConnaughey TA (1989b) ^{13}C and ^{18}O disequilibrium in biological carbonates, II, In vitro simulation of kinetic isotope effect. Geochim Cosmochim Acta 53:163–171

McConnaughey TA, Burdett J, Whelan JF, Paull CK (1997) Carbon isotopes in biological carbonates: Respiration and photosynthesis. Geochim Cosmochim Acta 61:611–622

O'Neil JR, Clayton RN, Mayeda TK (1969) Oxygen isotope fractionation in divalent metal carbonates. J Chem Phys 31:5547–5558

Pascual J, Flos J (1984) Meterologia i oceanografia. In: Ros J, Olivella I, Gili J (eds) Els sistemes naturals de les illes Medes. Institut d'Estudis Catalans, Arxius de la Seccio de Ciencies, LXpXIII, Barcelona, pp 75–114

Paull CK, Martens CS, Chanton JP, Neumann AC, Coston J, Jull AJT, Toolin LJ (1989) Old carbon in living organisms and young $CaCO_3$ cements from abyssal brine seeps. Nature 341:166–168

Pico (ed) (1990) Climatological atlas of the western Mediterranean. ENEA, Rome

Quay PD, Tilbrook B, Wong CS (1992) Oceanic uptake of fossil fuel CO_2: carbon-13 evidence. Science 256:74–79

Rhoads DC, Lutz RA (1980) Skeletal growth of aquatic organisms: biological records of environmental change. Plenum, New York

Richardson CA, Kennedy H, Duarte CM, Kennedy P, Proud SV (1999) Age and growth of the fan mussel *Pinna nobilis* from south-east Spanish Mediterranean seagrass (*Posidonia oceanica*) meadows. Mar Biol 133:205–212

Romanek CS, Grossman EL (1987) Stable isotope profiles of *Tridacna maxima* as environmental indicators. Palaios 4:402–413

Romanek CS, Grossman EL, Moorse JW (1992) Carbon isotopic fractionation in synthetic aragonite and calcite: effects of temperature and precipitation rate. Geochim Cosmochim Acta 56:419–430

Swart PK (1981) The carbon isotope composition of organic material in coral skeletons and its effect on early diagenesis. Proc 4th Int Coral Reef Symp (Manila) 2:87–90

Tanaka N, Monaghan MC, Rye DM (1986) Contribution of metabolic carbon to mollusc and barnacle shell carbonate. Nature 320:520–523

Urey HC (1947) The thermodynamic properties of isotopic substances. J Chem Soc 1:562–581

Watanabe T, Oba T (1999) Daily reconstruction of water temperature from oxygen isotopic ratios of a modern *Tridacna* shell using a freezing microtome sampling technique. J Geophys Res 104(C9):20667–20674

Weidman CR, Jones GA, Kyer (1994) The long-lived mollusc *Arctica islandica*: a new palaeoceanographic tool for the reconstruction of bottom temperatures for the continental shelves of the northern North Atlantic Ocean. J Geophys Res 99:18305–18314