Ecological information and water mass properties in the Mediterranean recorded by stable isotope ratios in *Pinna nobilis* shells

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[1]   Sclerochronologic and stable isotope records in *Pinna nobilis* shells potentially record ecological and oceanographic information. *P. nobilis* is a subtidal bivalve adapted to live in a variety of environments in the Mediterranean. We hypothesized that stable isotope ratios (δ^{18}O and δ^{13}C) and growth increment patterns from individuals living in different environments serve as ecological indicators. Using a new methodology for calcite sampling, we (1) identified annual growth features (nacre tongues) and (2) compared monthly resolved variations in δ^{18}O and δ^{13}C values and calcification temperatures recorded in animals located above and below the thermocline (16 and 30 m depth). The specimens from 16 m showed more negative δ^{18}O values than the specimen from 30 m, likely reflecting differences in salinity. The specimens from 30 m recorded δ^{13}C values less positive than the specimens from 16 m, which we interpreted as an ontogenetic effect observed in previous studies. Estimated calcification temperatures were offset relative to measured water temperature by ~6.1°C (~1.4‰). This finding is evident in earlier proxy studies of *P. nobilis*, although it was not discussed in those studies. Using the seasonal pattern of δ^{18}O and δ^{13}C values, we demonstrated that nacre tongues are deposited annually and that their formation is independent of temperature. Food availability rather than temperature may control nacre tongue formation. An alternative explanation for nacre tongue formation is gonad maturation during spring. Our findings support the idea that sclerochronology in *P. nobilis* can be used to reconstruct environmental, ecological, and climate archives of the Mediterranean.

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1. Introduction

[2]   Environmental characteristics are imprinted in biogenic carbonates at the time of their formation. The accretionary growth of bivalve shells makes them exceptional recorders of environmental parameters at subannual scales [*Richardson*, 2001]. Moreover, bivalves provide rich environmental and climate records in places where traditional archives, such as corals or foraminifera from marine sediment cores, do not exist [*Jones and Allmon*, 1995; *Goodwin et al.*, 2001; *Chauvaud et al.*, 2005; *Aguirre et al.*, 2006; *Fenger et al.*, 2007; *Wanamaker et al.*, 2007]. For example, sclerochronology (growth increment analysis) and oxygen isotope ratios (δ^{18}O) in the shells of estuarine bivalves have been used to estimate river discharge prior to the damming of the

Colorado River [*Schöne et al.*, 2002; *Dettman et al.*, 2004]. *Schöne et al.* [2005a] used oxygen and carbon isotope ratios (δ^{13}C) from the shells of *Arctica islandica* to estimate annual cycles in temperature and food availability, and decadal cycles of North Atlantic Oscillation (NAO) forcing near Iceland during the period 1495–1533 AD. Previous studies of *Pinna nobilis* shell geochemistry used δ^{18}O values to reconstruct seasonality of sea surface temperature (SST) in the western Mediterranean Sea [*Kennedy et al.*, 2001; *Freitas et al.*, 2005]. However, proxy records from shells of bivalves adapted to live under a wide range of environmental conditions or that may be influenced by vital effects can be difficult to interpret. Therefore, the calibration of proxy records (particularly, sclerochronologic and isotopic data) with environmental variables is necessary to reconstruct environmental, ecological, and climate information.

[3]   Traditionally, the study of sclerochronological records of marine invertebrate skeletons in the Mediterranean Sea has produced proxies with annual resolution with some exceptions, such as the scleractinian *Cladocora caespitosa*, where submonthly resolution was achieved [*Montagna et al.*, 2007] and the bivalve *P. nobilis*, which has been

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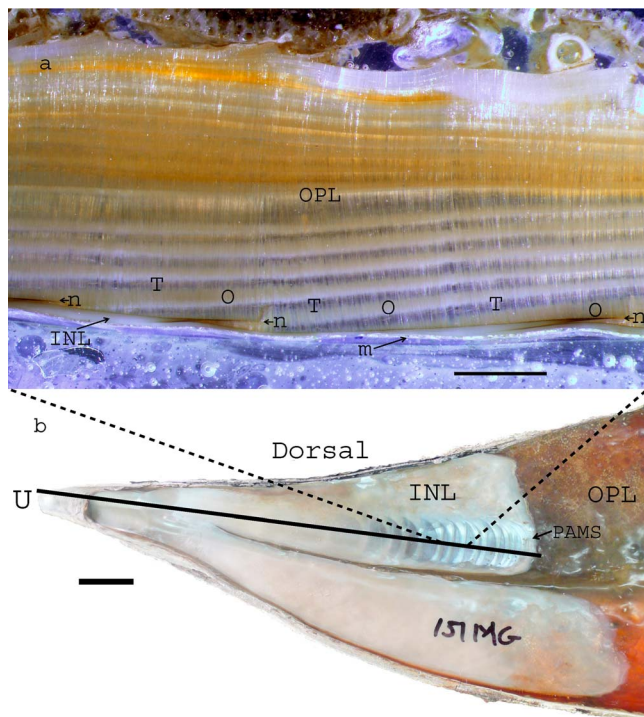
Figure 1. (a) Radial cross section showing shell layers and growth records of *P. nobilis* along the posterior adductor muscle scars (PAMS) and (b) location of radial sections in the inner surface of the valves. INL, inner nacreous shell layer; m, myostracum; n, nacre tongues; O, opaque layer; OPL, outer calcitic prismatic shell layer; T, translucent layer; U, umbo. Scale bar is 2 mm in Figure 1a and 3 cm in Figure 1b.

used to reconstruct Mediterranean SST at subannual scales [*Richardson et al.*, 1999; *Kennedy et al.*, 2001; *Freitas et al.*, 2005]. In *P. nobilis*, however, time averaging and the reduction of growth rates with ontogeny have lowered resolution of $\delta^{18}O$ records after the first 4.5 years of life [*Kennedy et al.*, 2001; *Freitas et al.*, 2005]. Improving the sampling methodology in this species would facilitate the reconstruction of longer and more precise chronologies at subannual scales in the Mediterranean basin.

[4] In the present study, we used isotopic and sclerochronologic techniques to develop a new microsampling method for the isotopic analysis of *P. nobilis* shells. We focused on the outer shell layer across the posterior adductor muscle scars (PAMS), which increased sampling resolution and allowed us to extend the microsampling range beyond the first 4.5 years of life. Using this new method, we tested the following hypotheses: (1) nacre tongues are deposited annually, (2) *P. nobilis* shells are deposited in isotopic equilibrium with seawater, and (3) isotopic records from animals living above and below the thermocline can be used to characterize water masses. We studied the calcite deposited within four consecutive nacre tongues in four shells from two populations located above (16 m) and below (30 m) the upper limit of the summer thermocline in Columbretes Islands, western Mediterranean. Our long-term

research goals are to reconstruct multidecadal climate archives using proxy records in *P. nobilis* shells.

## 2. Ecology and Sclerochronology of *Pinna nobilis*

[5] *P. nobilis* is one of the largest and fastest growing bivalves in the world with specimens larger than 100 cm in antero-posterior length [*Zavodnik et al.*, 1991]. This Mediterranean bivalve can live for several decades [*Vicente*, 1990] (http://pinnanobilis.free.fr/htm/accueil.htm, J. R. García-March, personal observation, 2010) and inhabits a variety of environments, including sandy and muddy sediments of shallow euryhaline-eurythermal coastal lagoons, seagrass meadows and boulder and maërl beds up to 60 m depth [*Templado*, 2002; *García-March*, 2003; *Zaouali*, 2003; *Katsanevakis*, 2005; *Rabaoui et al.*, 2008]. The valves are formed by a calcitic prismatic outer shell layer and an aragonitic nacreous inner layer. A thin aragonitic irregular simple prismatic myostracum is present under the muscle attachments and appears as a continuous thin veneer in radial sections through the PAMS (Figure 1). The calcitic layer of *Pinna* has a relatively high content of magnesium for a bivalve, from 2100 to 3650 ppm (0.9–1.5 mol% $MgCO_3$) [*Masuda and Hirano*, 1980]; however, these values indicate that their shells are low magnesium calcite.

[6] *García-March and Márquez-Aliaga* [2007] showed that the calcitic layer of *P. nobilis* has clear growth increments in radial sections across the PAMS and suggested that these were formed annually. Three components define each growth increment: a nacre tongue embedded within the calcite in the posterior direction and a couplet of translucent and opaque (or dark and light) layers (Figure 1). Translucent and opaque layers are visible under transmitted light, whereas dark and light layers are visible under reflected light. We will refer to translucent versus opaque layers throughout the rest of this paper.

[7] The occurrence of clear growth increments in the shell and the longevity of *P. nobilis* potentially allows for the construction of master chronologies that extend beyond the lifetime of an individual animal, such as the master chronologies derived from *A. islandica* shells [*Scourse et al.*, 2006; *Schöne and Fiebig*, 2009; *Butler et al.*, 2010]. Furthermore, the shell of *P. nobilis* may serve as a valuable recorder of environmental variables under a wide range of conditions and over relatively long time intervals.

3. Regional Setting

[8] The Columbretes Islands are located in the northwestern Mediterranean on the outer Ebro continental shelf 56 km off Castellón coast (northeast Spain) within a volcanic field that covers an area of 90 km × 40 km at 80–90 m water depth [*Muñoz et al.*, 2005]. The archipelago is composed of four main islets, L'Illa Grossa, La Ferrera, La Foradada and El Carallot, which were formed around 1 to 0.3 mya [*Muñoz et al.*, 2005]. The largest island is L'Illa Grossa, a volcanic cone that has been invaded by marine water after erosion by marine processes at its northeastern side [*Aparicio and García*, 1995] (Figure 2). The general water circulation in the zone is dominated by the surface Northern Current which carries water from the Gulf of Lion
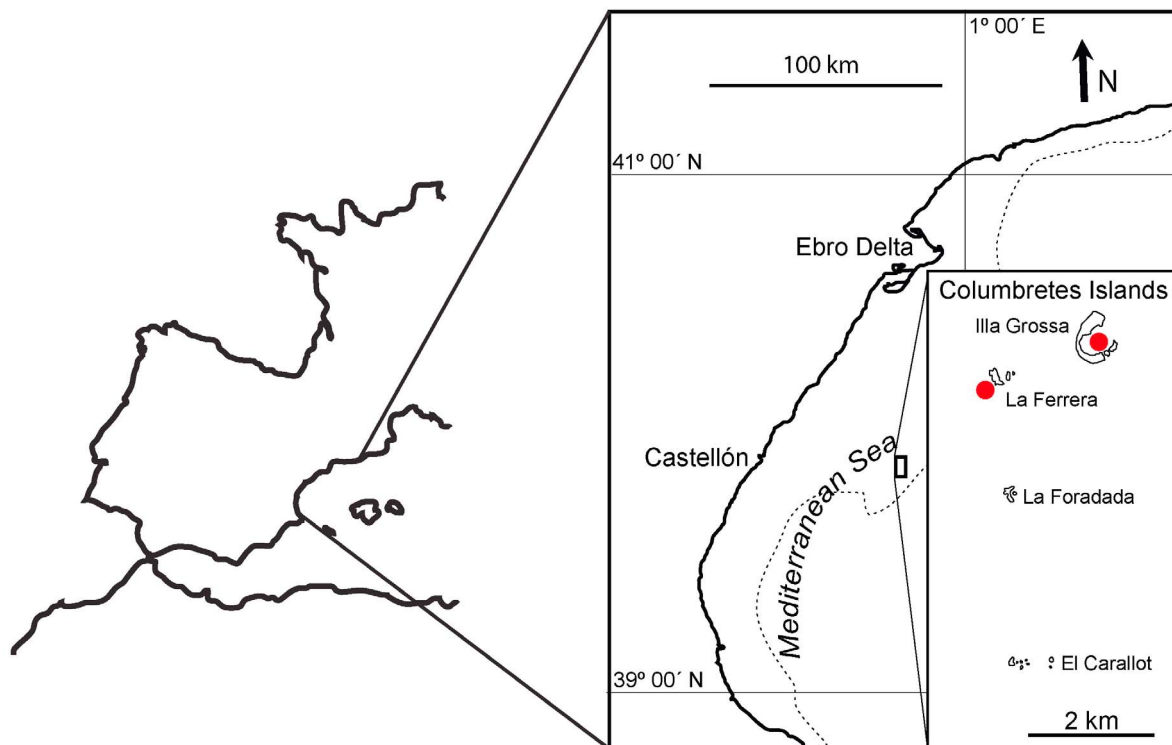
Figure 2. Map of the study area near the Columbretes Islands, western Mediterranean Sea. Red dots indicate the locations where *P. nobilis* specimens were collected. Two specimens were sampled in L'Illa Grossa at 16 m depth (specimen numbers A16 and B16), and another two were sampled in La Ferrera Island at 30 m depth (specimen numbers C30 and D30). Dotted line represents the margin of the continental shelf.

southward along the continental shelf [*Pinot et al.*, 2002]. The surface waters (also known as Modified Atlantic Water) penetrate down to 150–300 m. Salinity is around 38.0–38.3 psu (practical salinity units) in the north of the western Mediterranean Sea and have a mean temperature below the mixed layer of usually 14–15°C. The winter intermediate water (ranging from 12.5 to 13.0°C and 38.1–38.3 psu) is formed on the continental shelves of both the Gulf of Lion and the Balearic Sea and is found below the surface waters [*Millot*, 1999]. The effects of Ebro River discharge can be distinguished as an additional low salinity, continental influenced water (<37.8 psu) in the surface layer and is not deeper than 30 m. Its extension and presence depends on the variability of both the river discharge and the local thermal stratification [*Salat et al.*, 2002].

4. Methods

### 4.1. Water Temperature and Salinity Samples

[9] To characterize water temperature ranges and thermal gradients between 16 and 30 m depth at Columbretes Islands, we used direct temperature data routinely collected at L'Illa Grossa since 2005 (data provided by Columbretes Islands Marine Reserve). Weekly to fortnightly profiles (0–85 m depth) were available from 12 January 2005 to 4 April 2009. Additional data from Tidbit temperature loggers was obtained from 4 June 2007 to 19 March 2009 at hourly intervals at 15 and 30 m depth. Temperature values were

smoothed using a 30 day moving average to simulate the time averaging of the shell microsampling. Salinity for the zone was observed to range between 36.54 to 38.40 psu between 15 and 30 m, according to the maximum and minimum values reported for the water masses around Columbretes Islands and values from *Brasseur et al.* [1996] and *Sabates et al.* [2007, 2009]. Further salinity data for the zone was obtained from MEDATLAS database [*MEDAR-Group*, 2002] for stations located around Columbretes Islands within 39°30′N to 40°9.75′N and 0°10′E to 0°59.3′E. The salinity samples were available from 1983 to 1993 at 14 stations and for scattered months (February, April, May, June, July, August and October).

4.2. Shell Collection and Sampling

[10] Two shells were collected in Puerto Tofiño within the flooded caldera of L'Illa Grossa at 16 m depth (specimens A16 and B16), and another two specimens were collected in La Ferrera at c. 1400 m to the southwest of L'Illa Grossa at 30 m depth (specimens C30 and D30) (Figure 2). Three animals (A16, B16 and C30) were tagged in 2005 and their lengths measured periodically. The animals died some time between late spring 2007, the last time measured alive, and early spring 2009, when the shells were collected. A fourth untagged shell (specimen D30) was collected from La Ferrera in July 2010, and its exact date of death is unknown. We estimated that the animal died no more than 2 or 3 years before collection (i.e., between 2007 and 2008). At the time
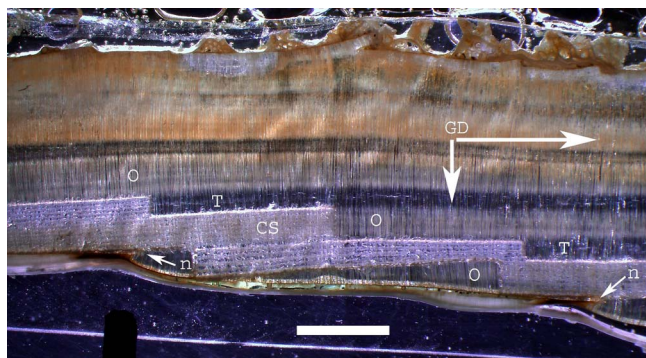
Figure 3. Photomicrograph of a cross section of *Pinna nobilis* shell along the posterior adductor muscle scars (PAMS) showing location of calcite microsamples (cs) between consecutive nacre tongues (n) of specimen A16. In all shells, the samples were taken following the growth increments observed under transmitted light. A couplet of translucent and opaque layers (T and O) is repeated between each other nacre tongue. The anterior part of the shell is to the left, and the inner shell layer is at the bottom. GD, growth direction. Scale bar is 2 mm.

of collection, the shell was still in life position. Other taphonomic indicators supporting our estimated time of death included: (1) moderate bioerosion, (2) few epibionts growing on the inside of the shell, and (3) the presence of some nefrolites [*Reid and Brand*, 1989] in the interior of the shell. Based on size and external appearance, all animals were adults with an estimated age above 10 years old. Size at death was A16 = 50.5, B16 = 58.4, C30 = 66.5 and D30 = 67.1 cm. Each specimen was set in a metal-based epoxy resin and cut in 8 cm radial sections along the PAMS observed in the dorsal nacre lobe (Figure 1). A 2 mm thick section mounted to a glass microscope slide was cut from each piece using a Buehler Isomet low-speed saw and polished down to 1 μm diamond suspension grit (Buehler).

[11] High-resolution microsampling for isotopic analysis was achieved using a Merchantek micromill. To avoid the mixing between consecutive growth layers and between calcite layers and aragonite nacre tongues, microsampling paths followed the growth lines formed by the opaque and translucent layers between nacre tongues from umbo to growing edge (Figure 3). Shell cross sections were microsampled at approximately 50–100 μm spacing (10–12 samples per year) across the first to fourth nacre tongues of specimens A16 and B16, the tenth to thirteenth nacre tongues of specimen C30, and the seventh to tenth nacre tongues in specimen D30. Note that the nacre tongue numbers do not correspond to the age of the individual because nacre tongues during the first few years of life are not visible in the shell [*García-March and Márquez-Aliaga*, 2007]. Therefore, the material sampled corresponds to ages 5–7 in shell A16, 4–6 in shell B16, 13–15 in shell C30, and 11–13 in shell D30. This sampling strategy allowed us to achieve nearly monthly resolution. Each digitized drilling path produced approximately 50 μg of carbonate powder for isotopic analysis. With this sampling strategy, it was possible to: (1) compare similar growth periods between

animals of the same population, (2) check for ontogenetic variation in the deposition of the nacre tongues, and (3) compare isotopic records of animals living in different environments above (A16 and B16) and below (C30 and D30) the summer thermocline.

4.3. Isotopic Analysis and Growth Temperature Calculation

[12] Oxygen and carbon isotope ratios of carbonate powdered samples were measured using an automated carbonate preparation device (Kiel-IV) coupled to a gas-ratio mass spectrometer (Finnigan MAT 253). Powdered samples were reacted with dehydrated phosphoric acid under vacuum at 70°C for 1 h. The isotope ratio measurement was calibrated based on repeated measurements of NBS-19 (National Bureau of Standard) and NBS-18. The precision of the measurement was >0.1‰ (1σ) based on monthly analyses of standards in excess of n = 180. The results were reported in per mil units (‰) relative to the VPDB (Vienna Pee Dee Belemnite) standard.

[13] We estimated calcification temperature from $\delta^{18}O_{SHELL}$ values using the equilibrium fractionation equation for calcite and water [*Friedman and O'Neil*, 1977]

$$1000 \ln \alpha = 2.78 \times 10^6 / T^2 - 2.89 \quad (1)$$

where T is temperature in Kelvin and α is the fractionation factor between calcite and water. We chose this equation over other linear or quadratic temperature equations derived from species-specific data because no correction is required to adjust the water and carbonate $\delta^{18}O$ values to the different scales on which they are measured (see *Dettman et al.* [1999, p. 1053] for a more detailed discussion). To account for the average 1.2 mol% $MgCO_3$ in *Pinna* shells, we used the 0.17 ± 0.02‰ per mol% $MgCO_3$ relation reported by *Jimenez-Lopez et al.* [2004] (the previous relation of *Tarutani et al.* [1969] was 0.06‰ per mol% $MgCO_3$) and added 0.2‰ to our $\delta^{18}O_{SHELL}$ values. This results in a decrease in estimated temperature of ~0.9°C. The relationship between α and δ is

$$\alpha = (\delta_{SHELL} + 1000)/(\delta_{WATER} + 1000) \quad (2)$$

where δ is expressed relative to VSMOW (Vienna-Standard Mean Ocean Water). Predicted $\delta^{18}O$ values calculated from equation (1) were converted from the VSMOW to VPDB scale using the following equation reported by *Coplen et al.* [1983] and *Gonfiantini et al.* [1995]:

$$\delta^{18}O_{VPDB} = \left(\delta^{18}O_{(VSMOW)} - 30.91\right)/1.03091 \quad (3)$$

[14] $\delta^{18}O_{WATER}$ values were not available for the corresponding periods sampled in the shells, but can be estimated from salinity values. The climatic regime of the Mediterranean is characterized by an excess of evaporation relative to the amount of freshwater input, and salinity is mainly a function of evaporation. We used the following $\delta^{18}O_{WATER}$-salinity relationship for western Mediterranean surface waters (R = 0.88 [*Pierre*, 1999]):

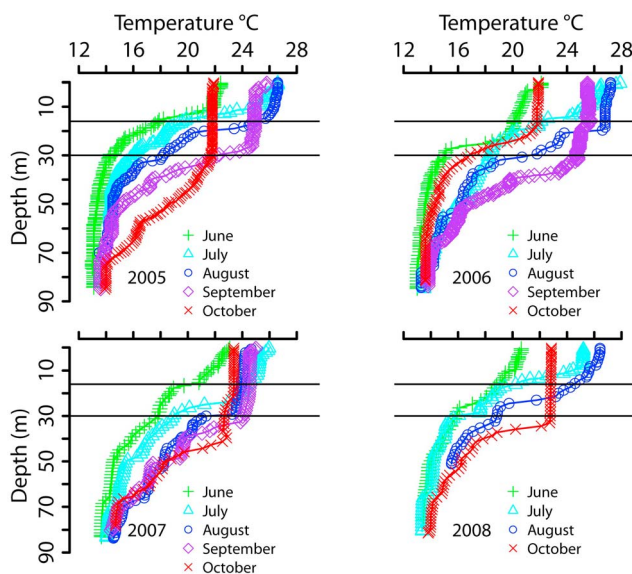$$\delta^{18}O_{WATER} = 0.25S - 8.23 \quad (4)$$
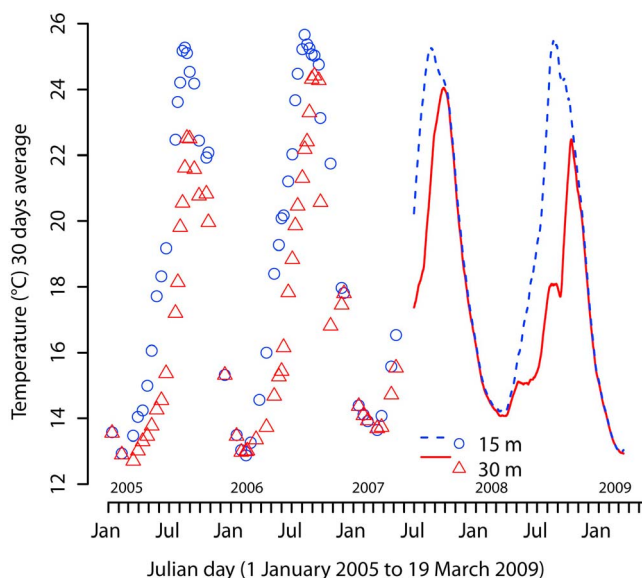
Figure 4. Plots of temperature recorded at 15 and 30 m depth at L'Illa Grossa in Columbretes Islands. Data from January 2005 to May 2007 have been extracted from the temperature profiles and are weekly to fortnightly. Temperature measurements were made hourly from June 2007 to March 2009. Data have been smoothed with a 30 day moving average.


Figure 5. Temperature profiles from 0 to 85 m depth obtained at L'Illa Grossa in Columbretes Islands from June to October of years 2005–2008. The 16 and 30 m depths have been highlighted with horizontal lines. The top of the thermocline is always above 30 m except for late August and September/October, when it is a few meters below 30 m. September 2008 data are missing.

where S is salinity. We assumed a $\delta^{18}O_{WATER}$ value of +1.26‰ (VSMOW); see section 6.3 for a more detailed explanation.

5. Results

5.1. Water Temperature and Salinity in Columbretes Islands

[15] Water temperatures measured from January 2005 to March 2009, smoothed with a 30 day moving average, range from 12.88 to 25.66°C at 15 m and from 12.71 to 24.42°C at 30 m (Figure 4 and Table 1). Average differences in the

warmest and coldest water temperatures at 15 and 30 m for the 4 years were 1.64 ± 0.80°C and 0.06 ± 0.14°C (mean ± SD). In all years, the thermocline starts to form in early spring and persists until late September to early October, when the heat loss of the water reduces the temperature gradients until the thermocline disappears. The top of the thermocline was above 30 m most of the time, except for some periods in August or September, when it was situated up to 35 m (Figure 5). The warming of the water mass at 30 m lags the warming at 16 m, and maximum temperatures at 30 m are only achieved in late summer to early autumn, coinciding with a cooling of surface waters and the

Table 1. Ranges of Instrumental and Calcification Temperatures (°C) in Columbretes Islands From January 2005 to March 2009 and Specimens A16, B16, C30, and D30[a]

	Instrumental Temperature						Calcification Temperatures A16					Calcification Temperatures B16				
	2008	2007	2006	2005	Average	SD	2004	2003	2002	Average	SD	2003	2002	2001	Average	SD
	15 m															
Maximum	24.35	24.98	25.66	25.27	25.30	0.34	26.1	26.5	26.8	26.5	0.4	23.6	25.3	23.8	24.2	0.9
Minimum	14.19	13.69	12.88	12.94	13.17	0.45	15.6	16.8	16.5	16.3	0.6	15.9	16.0	15.9	15.9	0.1
Range	10.16	11.29	12.78	12.33	12.13	1.17	10.5	9.7	10.3	10.2	0.4	7.7	9.3	7.9	8.3	0.9

	Instrumental Temperature						Calcification Temperatures C30					Calcification Temperatures D30				
	2008	2007	2006	2005	Average	SD	1997	1996	1995	Average	SD	2007	2006	2005	Average	SD
	30 m															
Maximum	22.48	24.05	24.42	22.52	23.66	1.01	22.1	21.9	20.1	21.4	1.1	23.5	23.5	22.1	23.0	0.8
Minimum	14.07	13.64	12.98	12.71	13.11	0.48	14.3	13.4	13.4	13.7	0.5	14.8	15.0	15.2	15.0	0.2
Range	8.41	10.41	11.44	9.81	10.55	1.26	7.8	8.5	6.7	7.7	0.9	8.7	8.5	6.9	8.0	1.0

[a]Calcification temperatures were calculated with equation (1) using $\delta^{18}O_{WATER} = 1.26$‰ and corrected for the offset of 6.1°C. SD, standard deviation. Note that specimens A16 and B16 lived at 16 m depth.
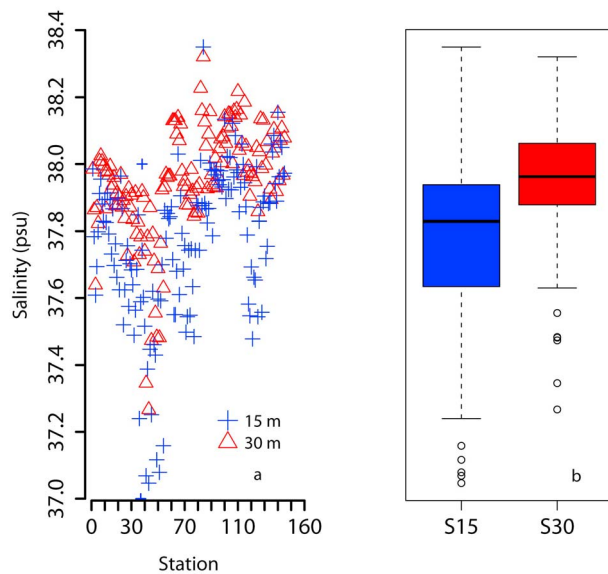
Figure 6. Salinity data obtained from MEDATLAS database [*MEDAR-Group*, 2002] for different CTD casts around Columbretes Islands. (a) Data from stations at 15 (blue pluses) and 30 (red triangles) m depth. (b) Boxplot of data showing means and standard deviations (2 σ) for salinity data at 15 (S15) and 30 (S30) m depth. Open circles are extreme values. Stations show all simultaneous salinity casts at 15 and 30 m within 39°30′N to 40°9.75′N and 0°10′E to 0°59.3′E present in the database. Differences in salinities between the two depths are significant with a p value < 0.01. Salinity is lower at 15 versus 30 m depth in most of the stations.

disappearance of the thermocline (Figures 4 and 5). Salinity values from MEDATLAS database range from 37.05 to 38.35 psu, with a mean and SD of 37.95 ± 0.17 and 37.77 ± 0.23 psu at 15 and 30 m, respectively. Despite the fragmentary nature of the data, statistical analysis confirmed that salinities are different in the two depths (p value < 0.01, n = 141). For most of the stations, salinity was lower at 15 m, as expected due to the occurrence of continental influenced water in the upper < 30 m water layer (Figure 6).

5.2. Oxygen and Carbon Isotope Ratios

[16] Oxygen isotope ratios in all specimens are highly positive, most of them above 0‰, with marked annual cyclicity (Figure 7). The highest values form broad peaks, and the lowest values produce narrow valleys. Peaks and valleys in all shells are bounded by nacre tongues. Specimens A16 and B16 have similar $\delta^{18}O_{SHELL}$ values, ranging from −0.08 to 2.53‰ in shell A16 and from 0.25 to 2.46‰ in shell B16., Specimens C30 and D30 show more positive values, ranging from 0.97 to 3.08‰ in C30 and from 0.65 to 2.73‰ in D30 (Figure 7 and Table 2).

[17] Carbon isotope ratios follow an annual sinusoidal pattern in all years recorded in specimens B16, C30 and D30, and in the last 2 sampled years in specimen A16. $\delta^{13}C$ values range from 0.93 to 2.46‰, 0.88 to 2.36‰, 0.53 to 1.77‰, and 0.68 to 1.82‰ in specimens A16, B16, C30 and D30, respectively (Table 2). The average value between

nacre tongues decreases with increasing age (Figures 7 and 8).

6. Discussion

6.1. New Methodology for Geochemical Sampling in *Pinna nobilis*

[18] Our new sampling methodology enabled us to decipher the isotopic time series recorded in the shells with higher resolution (e.g., monthly to submonthly) across a wider range of growth years than attainable in earlier studies of *P. nobilis* [*Kennedy et al.*, 2001; *Freitas et al.*, 2005]. Microsampling along the opaque and translucent layers between nacre tongues allowed us to reduce mixing of material among consecutive coeval samples. Geochemical sampling of *P. nobilis* shells has been traditionally performed on the external shell surface. The external shell surface is strongly abraded in adults [*García-March and Márquez-Aliaga*, 2007; *García-March et al.*, 2008] and bioeroded by encrusting organisms [*Zavodnik*, 1967; *Corriero and Pronzato*, 1987; *Giacobbe*, 2002; *Cosentino and Giacobbe*, 2008]. Furthermore, sampling along the external shell surface increases the probability of mixing layers that did not form coevally (i.e., time averaging) because in adult Pinnids shell length and thickness increase slowly by deposition of long, thin sheets of new material [*Yonge*, 1953]. Our new sampling method avoids the potential contamination problems and reduces time averaging inherent in the traditional method.

6.2. Seasonal Variations in Oxygen and Carbon Isotopes

[19] Oxygen isotope ratios reveal seasonal variation. Broad peaks (i.e., high values) reflect growth during cold months, whereas narrow valleys (i.e., low values) represent growth during warm months (Figure 7). We interpret this pattern as fast growth during cold months (broad peaks) and slow growth during warm months (narrow valleys). Changes in seasonal growth patterns have been attributed to temperature and/or food availability [*Jones et al.*, 1989, 1990; *Jones and Quitmyer*, 1996; *Surge et al.*, 2001; *Schöne et al.*, 2005b]. For example, *Jones and Quitmyer* [1996] reported that the $\delta^{18}O$ record of a *Mercenaria mercenaria* shell from the cold-temperate biogeographic province along the eastern United States (Rhode Island) revealed that dark (translucent in transmitted light) increments coincided with high values (narrow valleys) during periods of slowed winter growth, whereas light (opaque in transmitted light) increments corresponded to lower values during fast growth the rest of the year. In contrast, they reported that the $\delta^{18}O$ record of *M. campechiensis* shells from the warm-temperate biogeographic province along the eastern United States (Florida and Georgia) showed the reverse pattern. The values and range of $\delta^{18}O$ values in specimens A16 and B16 are similar to those reported by *Kennedy et al.* [2001] and *Freitas et al.* [2005] for their *P. nobilis* specimens collected at c. 5 m depth in the western Mediterranean (Table 2). *Kennedy et al.* [2001] and *Freitas et al.* [2005] observed growth cessation during winter, which is in apparent contradiction to the growth patterns observed in our isotopic records. A possible explanation for this apparent contradiction is that our pinnids also ceased growing below a
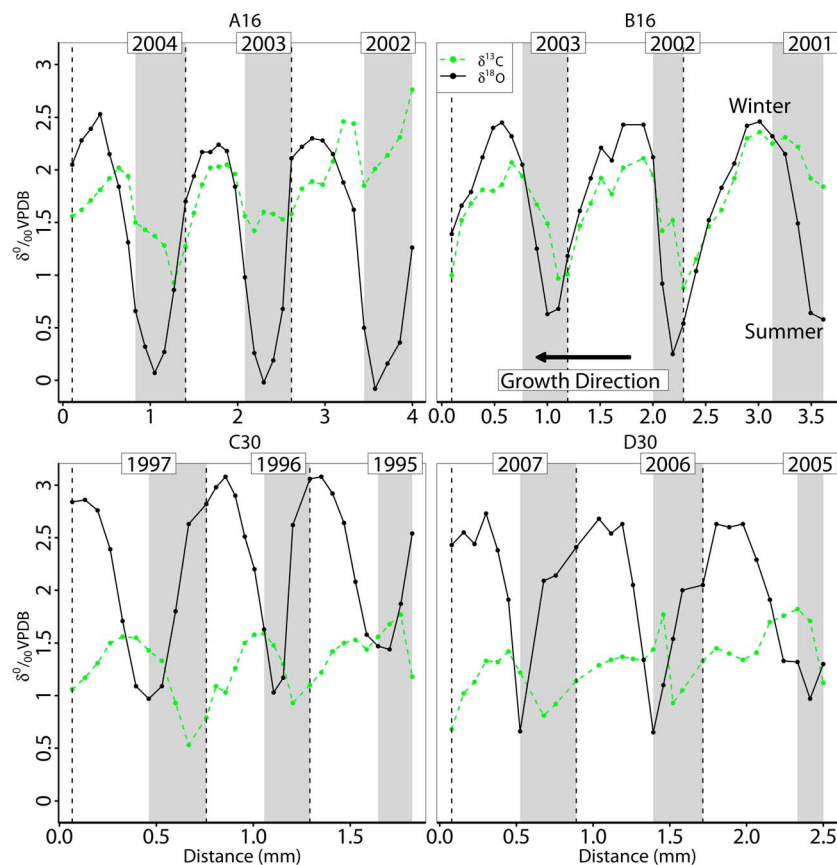
Figure 7. The $\delta^{18}O$ and $\delta^{13}C$ values of *P. nobilis* shell calcite from the Columbretes Islands versus distance (mm). Samples were taken along the inner record of the posterior adductor muscle scar, from radial sections of the shell. Measurements were taken orthogonally to the inner layer of the shell, starting in the innermost (younger) layer. Growth direction is from right to left, so the younger material is to the left. Black symbols (continuous line) are $\delta^{18}O$ values; green symbols (dashed line) are $\delta^{13}C$ values; vertical dotted lines are location of nacre tongues. Gray vertical bars represent the extent of the translucent layer.

minimum threshold temperature in winter, but the growth rate in early spring rapidly increased with warming water and food availability. This hypothesis needs further testing, but has been observed in other bivalve species in the Mediterranean [*Gangnery et al.*, 2004; *Peharda et al.*, 2007]. *Kennedy et al.* [2001] also reported an ontogenetic trend in their $\delta^{18}O$ values where annual amplitudes and the most positive winter $\delta^{18}O$ values decreased with age. Contrary to their previous study, there was no clear ontogenetic trend in our data. This may result from the shorter growth period sampled (our study: years 4–6 and 5–7 in specimens A16 and B16 and years 13–15 and 11–13 in specimens C30 and D30; their study: years 1–10.5 in one individual and 1–4.5 in other). Alternatively, *Freitas et al.*'s [2005] observation of an apparent ontogenetic trend may be an artifact of their sampling methodology. Future work will test the hypothesis of ontogenetic trends using our new sampling method.

[20] In addition to seasonal patterns, our isotopic data revealed differences relative to depth. $\delta^{18}O$ values showed more positive values and lower amplitudes in the shells collected at 30 m (i.e., below the thermocline) than the shells from 16 m (above the thermocline; Table 2). We attribute the more positive values in specimens C30 and D30 to

higher $\delta^{18}O_{WATER}$ values, probably associated to higher salinities below the thermocline. Historic data confirms the occurrence of significant higher salinities at 30 m (p value < 0.01, n = 141) [*MEDAR-Group*, 2002]. The smaller

Table 2. Ranges of $\delta^{18}O$ and $\delta^{13}C$ Values of Shells A16, B16, C30, and D30 (Our Study) and Ag4a and V4b ([*Freitas et al.* [2005] Study)

Specimen	Minimum	Maximum	Average	SD	Range	Years Sampled	Age (Years)
			$\delta^{18}O$ (‰$_{VPDB}$)				
A16	−0.08	2.53	1.39	0.9	2.61	5–7	9–10
B16	0.25	2.46	1.67	0.7	2.21	4–6	9–10
C30	0.97	3.08	2.19	0.7	2.11	13–15	26–27
D30	0.65	2.73	1.98	0.6	2.08	11–13	13–14
Ag4a	−0.24	2.38	1.04	0.8	2.62	1–10.5	10.5
V4b	−0.29	2.41	1.21	0.7	2.7	1–4.5	4.5
			$\delta^{13}C$ (‰$_{VPDB}$)				
A16	0.93	2.46	1.79	0.4	1.53	5–7	9–10
B16	0.88	2.36	1.74	0.4	1.48	4–6	9–10
C30	0.53	1.77	1.31	0.3	1.24	13–15	26–27
D30	0.68	1.82	1.31	0.3	1.14	11–13	13–14
Ag4a	0.74	3.05	1.93	0.5	2.31	1–10.5	10.5
V4b	1.4	3.02	2.55	0.4	1.62	1–4.5	4.5
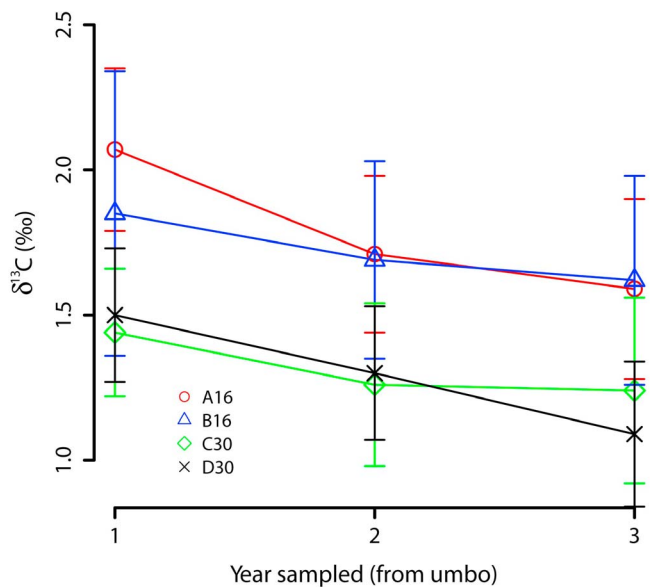
Figure 8. Average $\delta^{13}C$ values of isotopic records between nacre tongues in shells A16, B16, C30, and D30. Vertical bars represent ±1 standard deviation. The values are always less positive each consecutive year for all shells. Shells C30 and D30, where an older time sequence was sampled, show less positive values than A16 and B16.

amplitude is likely explained by the reduced temperature range at 30 m than at 16 m. Measured temperature data above and below the thermocline reflects reduced amplitudes at 30 m relative to 16 m (12.88 to 25.66°C at 15 m and 12.71 to 24.42°C at 30 m; an average difference of 1.64 ± 0.80°C between the two depths in the warmest months). In section 6.3, we discuss more fully the implications of salinity and temperature differences with depth relative to $\delta^{18}O$ values and calculated calcification temperatures.

[21] Like oxygen, carbon isotope ratios form broad peaks (i.e., higher values) and narrow valleys (i.e., lower values). The amplitude of the annual records between nacre tongues is similar to the annual variation observed by *Kennedy et al.* [2001] and *Freitas et al.* [2005] in two *P. nobilis* from the western Mediterranean. In our shells, however, each cycle shows a clearer pattern with pronounced peaks and valleys. Older portions of specimens C30 and D30 (years 13–15 and 11–13, respectively) showed less positive values and smaller amplitudes than younger portions of specimens A16 and B16 (years 5–7 and 4–6, respectively; Table 2). This finding, together with decreasing $\delta^{13}C$ values within individuals (Figure 8), likely reflects an ontogenetic pattern, where $\delta^{13}C$ values decrease with an individual's age. This observation is consistent with the ontogenetic trend toward lower $\delta^{13}C$ values with age documented by *Kennedy et al.* [2001] and *Freitas et al.* [2005]. As suggested by *Kennedy et al.* [2001], the ontogenetic trend toward more negative values may be caused by increasing incorporation of metabolic carbon into the shell with increased age. The $\delta^{13}C$ values in the shells of *P. nobilis* at our two stations seem to be less affected by water depth than the $\delta^{18}O$ values. Some authors have related $\delta^{13}C_{SHELL}$ values to concentrations of chlorophyll a and phytoplankton [*Schöne et al.*, 2005b] and these were expected to be similar by proximity in our two stations.

6.3. An Offset From Isotopic Equilibrium and the Effect on Reconstructed Calcification Temperatures

[22] Using equation (4) and the average salinity of 37.85 psu, we calculated a $\delta^{18}O_{WATER}$ value of 1.26‰, which was used to estimate calcification temperatures. The average salinity was used to calculate $\delta^{18}O_{WATER}$ because we did not have salinity measurements for the sampling period in the shells. For comparison, we used the minimum (36.54 psu) and maximum (38.40 psu) salinity reported for 15 and 30 m depth to calculate the possible range of $\delta^{18}O_{WATER}$ values. Calcification temperatures ranged from 9.5 to 20.7°C, from 9.8 to 19.2°C, from 7.3 to 16.0°C and from 8.7 to 17.4°C in specimens A16, B16, C30 and D30, respectively (Figure 9 and Table 1). In all shells, summer and winter calcification temperatures are lower than measured water temperatures (Figures 4 and 9).The water column in the Mediterranean is homothermic in winter [*Deacon*, 1997], rarely dropping below 12.7°C in the coldest months [*Lacombe et al.*, 1985; *Salat et al.*, 2002]. Therefore, reconstructed winter temperatures for $\delta^{18}O_{WATER} = 1.26$‰ are approximately 3°C colder at 16 m and 4.5°C colder at 30 m than expected given the instrumental records.

[23] The temperature discrepancy may result from incorrect assumptions about our salinity estimate, and hence, our assumed $\delta^{18}O_{WATER}$ value. We applied the maximum reported salinity at 30 m (38.40 psu) and its corresponding $\delta^{18}O_{WATER}$ value (1.40‰) estimated from equation (4) to recalculate calcification temperatures for specimens C30 and D30. The recalculated minimum value is 7.8°C and 9.2°C, still far below measured winter water temperatures. The highest surface $\delta^{18}O_{WATER}$ value for the Mediterranean at 10 m depth reported by *Pierre* [1999] is 1.66‰ and corresponds to the saltier Levantine Basin having salinity of 38.98 psu. Therefore, it is not realistic to consider higher salinities or higher $\delta^{18}O_{WATER}$ values than 38.4 psu and 1.40‰, respectively, in the open waters around Columbretes Islands. To recalculate calcification temperatures at 16 m, we used a minimum winter salinity of 36.54 psu and its corresponding $\delta^{18}O_{WATER}$ value of 0.94‰ because surface water salinity above 30 m in the Columbretes Islands is influenced by freshwater discharge from Ebro River during the coldest months [*Salat et al.*, 2002]. The reduced salinity, and therefore lower $\delta^{18}O_{WATER}$ value, decreased the calcification temperatures of specimens A16 and B16 even further. The recalculated minimum winter calcification temperatures for these specimens (8.2 and 8.5°C, respectively) are similar to those of specimens C30 and D30 and too low relative to measured winter temperatures. Therefore, our assumptions about the $\delta^{18}O_{WATER}$ value cannot completely explain the discrepancy between estimated and measured temperatures.

[24] We have identified this discrepancy in previous stable isotope studies of *P. nobilis*; however, it was not discussed by the authors in these previous studies [*Kennedy et al.*, 2001; *Freitas et al.*, 2005]. Minimum and maximum calcification temperatures for the specimens studied by *Freitas et al.* [2005] are 10.3 and 10.1°C and 21.9 and 22.1°C, similar to our findings (note that they did not correct calcification temperatures by the content of Mg in the calcite, which would decrease their estimated temperatures by ~0.9°C). Local winter temperatures for their site were not
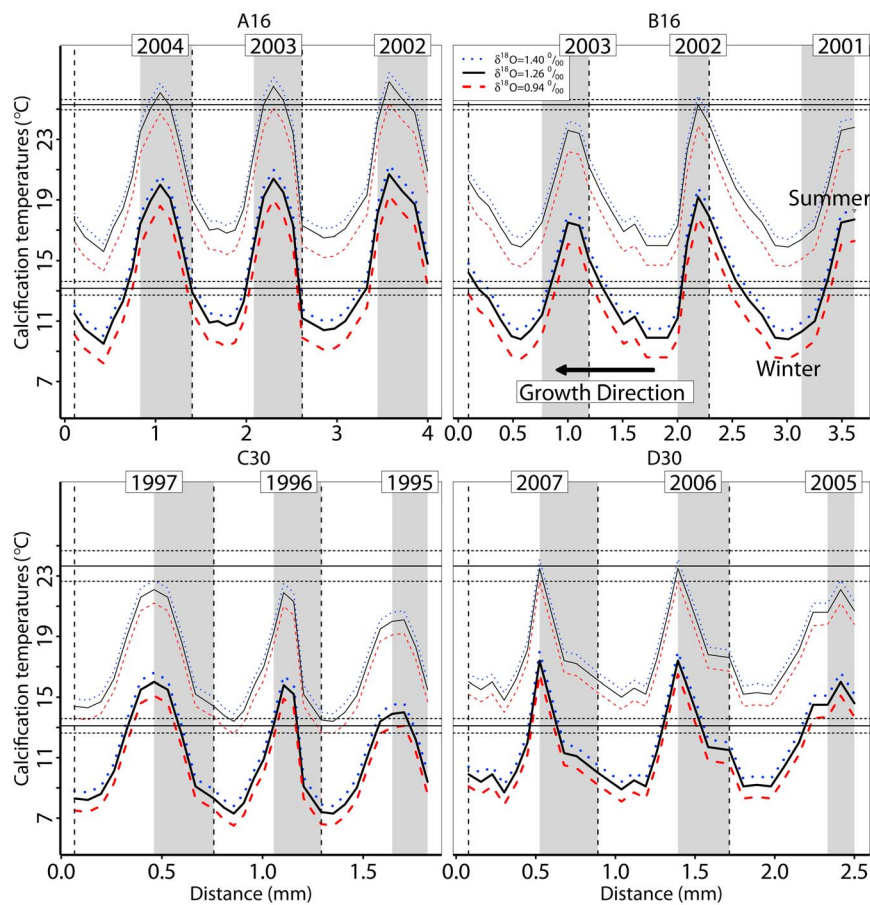
Figure 9. Calcification temperatures for the average (37.85 psu, 1.26‰, black continuous line), maximum (38.40 psu, 1.40‰, blue dotted line), and minimum (36.54 psu, 0.94‰, red dashed line) salinities and estimated $\delta^{18}O_{WATER}$ values in the water masses around Columbretes Islands versus distance (mm) (sampling as in Figure 7). Thick lines represent uncorrected temperatures whereas thin lines represent corrected temperatures with the estimated offset of 6.1°C. Note that minimum salinities are only observed in the surface layer <30 m depth, so mostly they will affect only specimens A16 and B16. Gray vertical bars represent the extent of the translucent layer. Horizontal lines represent average ± standard deviation of maximum and minimum instrumental temperatures measured from 2005 to 2009.

reported, but *Kennedy et al.* [2001] sampled the same shells and reported the minimum in situ temperature was between 14 and 15°C and the maximum averaged for August was 25.2 ± 0.9°C. The differences between calcification and instrumental temperatures are similar to those observed in our shells and imply the occurrence of an unreported consistent offset (i.e., a predictable vital effect) in *P. nobilis*. Offsets in calcification temperatures have been observed in other mollusks, such as limpets (see *Fenger et al.* [2007] for a detailed discussion). Because the offset was constant and predictable, several authors account for the offset by removing up to 1‰ from $\delta^{18}O_{WATER}$ [*Shackleton*, 1973; *Cohen and Tyson*, 1995] or $\delta^{18}O_{SHELL}$ values [*Shackleton*, 1973; *Cohen and Tyson*, 1995; *Fenger et al.*, 2007] in their respective paleotemperature equations.

[25] The shape of the calcification temperature curve from shell D30 provides additional quantitative information about the offset because the growth period of this shell overlaps with at least part of the measured temperature records (Figure 9). During 2005–2009, the lowest maximum instrumental temperature (which at 30 m depth occurs in late

summer early fall) was 22.48°C and the maximum calcification temperature of specimen D30 (assuming $\delta^{18}O_{WATER} = 1.40$‰) was 18.0°C. This observation can be explained by growth cessation during warm months, error in our $\delta^{18}O_{WATER}$ estimate, or a predictable vital effect. It is highly improbable that shell D30 ceased growing when water temperature was at or below 18°C. The Boreal mussels *Modiolus modiolus* and *Mytilus galloprovincialis* inhabiting the Mediterranean Sea, show optimum growth temperatures around 18°C and the first signs of thermal stress above 20°C and 24°C, respectively [*Read and Cumming*, 1967; *Anestis et al.*, 2007, 2008]. *P. nobilis* is a long-lived bivalve found from shallow euryhaline-eurythermal coastal lagoons to 60 m depth, so the thermal tolerance of *P. nobilis* should be above these temperatures. An error in our $\delta^{18}O_{WATER}$ estimate cannot completely explain this offset. To align estimated and measured temperatures would require a $\delta^{18}O_{WATER}$ value of ~2.8‰. Such a high $\delta^{18}O_{WATER}$ value has not been measured in the saltiest open waters of the Mediterranean (the highest measured value at 646 m depth was 1.68‰ [*Pierre*, 1999]). Therefore, a predictable vital

effect is the best explanation for the observed discrepancy between calcification and instrumental temperatures.

[26] The shape of the peaks recorded by the data loggers in years 2005–2007 provides another qualitative comparison to the reconstructed calcification temperatures in shell D30. Maximum temperature recorded by the data loggers in years 2005, 2006, and 2007 shows a very similar pattern to maximum calcification temperatures of shell D30 (Figures 4 and 9 and Table 1). The warmest month in the first year sampled in specimen D30 was ~1.4°C colder than the other 2 years, a difference of similar magnitude to the instrument record for 2005 and 2006. The maximum temperatures of the last 2 years were similar to each other as occurred in the instrument record for 2006 and 2007 (Figures 4 and 9). Strikingly, maximum calcification temperatures for the 3 years, were on average 6.1 ± 0.3°C (mean ± SD) colder than the measured temperatures (equivalent to ~1.4‰ in $\delta^{18}O_{SHELL}$). We are currently monitoring the water in Columbretes Islands for a 2 year period to constrain $\delta^{18}O_{WATER}$ values and to more precisely calculate the magnitude of the predictable offset from equilibrium values in *P. nobilis* shells.

6.4. *P. nobilis* as Climate Archives

[27] A goal of our research was to evaluate *P. nobilis* as a recorder of climate information. We observed that the amplitude of the calcification temperatures is smaller than the instrumental temperature records. The reduced range of calcification temperatures suggests that there is a threshold in the maximum and/or minimum temperature tolerances for *P. nobilis* growth, as observed in other *P. nobilis* from the western Mediterranean [*Kennedy et al.*, 2001; *Richardson et al.*, 2004; *Freitas et al.*, 2005]. The animals that lived at 30 m (C30 and D30), where temperature range is dampened relative to shallower depths, showed on average a reduced range of calcification temperatures than the animals that lived at 16 m (Table 1). Minimum instrumental temperatures in Columbretes Islands are nearly identical between depths at 15 and 30 m, and the reduced range at 30 m is owed to the reduced maximum temperatures at this depth (Figure 4). Therefore, the reduced range of calcification temperatures in specimens C30 and D30 relative to shells A16 and B16 may reflect the reduction in summer temperatures at 30 m relative to 15 m depth. Alternatively, an ontogenetic effect between older and younger individuals may influence maximum and/or minimum temperatures recorded in the shell (periods sampled in shells C30 and D30 were 7–10 years older than those in shells A16 and B16).

[28] Specimen D30 provides additional evidence to discriminate between these alternatives because its growth years partially overlap with the instruments records. The warmest calcification temperatures recorded in the 3 year growth period of specimen D30 capture the variability in the instrumental data for the period 2005–2007. In contrast, the coldest calcification temperatures throughout the growth period do not capture the winter variation in the instrumental data. Specifically, measured temperatures in 2005 have an amplitude of 9.81°C. The amplitude of the calcification temperatures for that year was 6.9°C (2.9°C less than measured temperatures). In 2006, the amplitude of measured temperatures was 11.44°C, and for that year the amplitude of calcification temperatures was 8.5°C (2.9°C less than

measured temperatures). In 2007, the amplitude of measured temperatures was 10.41°C, and for calcification temperatures was 8.7°C (1.7°C less than measured temperatures). In this year, the noticeable reduction in the amplitude difference between calcification and measured temperature was due to the warmer winter minima reducing the amplitude of instrumental temperatures (see Figure 4 and Table 1). We interpret this pattern as evidence that shell D30 grew during the warmest months and stopped growing before the minimum winter temperatures dropped below ~13–14°C. If we assume that the constant positive offset of ~1.4‰ is accurate resulting in a reduction of estimated calcification temperature by ~6.1°C, recalculated temperatures show that shell D30 stopped growing when temperatures were ~15°C, in good agreement with our interpretation based on amplitudes. We tested the potential of *P. nobilis* as a climate archive by comparing shell records with two extreme climatic events in the Mediterranean that occurred during the period of growth in specimens A16 and B16 (2001–2004).

[29] Year 2003 had the hottest summer in the last 30 years. Water temperatures were 1°C above mean and 3°C above maximum values, causing mass mortalities of many sessile invertebrate species [*Garrabou et al.*, 2009]. We do not have instrumental temperature for that period, but *Garrabou et al.* [2009] reported that maximum and average temperatures at 1 m depth at Columbretes Islands were 2.1°C and 0.8°C higher, respectively, compared to the period 1991–2006. Maximum temperature was 29.6°C relative to 27.5 ± 0.9°C during 1991–2006, and average summer temperature was 26.7°C relative to 25.9 ± 0.8°C from 1991 to 2006. Weather conditions during winter 2004–2005 were unprecedented in 40 years with five intense polar fronts reaching the Mediterranean coast of Spain and the Balearic Islands [*Lopez-Jurado et al.*, 2005]. The extremely dry, cold and windy atmospheric conditions resulted in the formation of atypical dense waters that were warmer and saltier than usual in winter 2004–2005 (12.99°C and 38.50 psu). The atypical dense waters penetrated down to 750 m in an uninterrupted cascading process lasting from late January to late March [*Canals et al.*, 2006; *Font et al.*, 2007]. This episode was reflected in the anomalously low values of chlorophyll a recorded for that winter, related to the increased vertical convection in the Catalan Basin and the reduced runoff of Ebro River [*Gordoa et al.*, 2008].

[30] The 2003 warm event is not reflected in the isotopic ratios of our shells. In fact, calcification temperatures of specimen B16 show abnormally cold temperatures for that summer, which may indicate some growth disturbance after such a hot event or the event was too brief at 16 m to be captured by our monthly to submonthy sampling resolution. Specimen A16, however, shows a positive spike in the $\delta^{18}O_{SHELL}$ values of winter 2004–2005 (Figure 7), which is coincident with the extremely dry and cold winter described by *Font et al.* [2007] and *Gordoa et al.* [2008]. Our measured temperatures for that winter do not show the presence of significantly colder waters than in previous winters at 16 m (Figure 4 and Table 1). Considering that winter 2004–2005 was exceptionally dry and windy, we hypothesize that the positive spike of $\delta^{18}O_{SHELL}$ values in shell A16 reflects an increased salinity in the surface layers of Columbretes Islands of about 1.5 psu during that winter relative to previous years. The increase in salinity may have been pro-

duced by the concomitant effects of increased evaporation and exceptionally low discharges of Ebro River.

[31] The amplitude and shape of the isotopic time series from our shells confirm that *P. nobilis* is a good paleo-temperature recorder at monthly scales for most of the record. As described by *Kennedy et al.* [2001] and *Freitas et al.* [2005], part of the annual variation in water temperature is lost. Our data indicates that *P. nobilis* grew during the entire summer, at least at 30 m depth, where maximum temperatures were not as warm as in surface waters (<10 m). Growth cessation was evident during the coldest winter months. Calcification temperatures were negatively offset by ~6.1 ± 0.3°C (~1.4‰ in $\delta^{18}O$) and the winter temperature threshold may be ~15°C. To better estimate the offset and the temperature threshold for shell growth, a calibration study with measured ambient temperature and $\delta^{18}O_{WATER}$ is necessary. Extending the sampling period to multiple years within a shell will allow us to constrain the winter temperature threshold for shell growth. Studying animals above and below the thermocline provides a novel approach to estimating decadal changes in water masses, which may be related to the North Atlantic Oscillation or the bidecadal oscillations in the Mediterranean marine climate [*Duarte et al.*, 1999].

6.5. Shell Growth and Ecology From Sclerochronology

[32] Our isotope results not only provide environmental and climate records, but also allow us to interpret shell growth records and ecological information. As suggested by *García-March and Márquez-Aliaga* [2007], our results demonstrate that the nacre tongues are deposited annually and, therefore, can be used to age animals. Previous studies have reported that temperature stress is the dominant factor controlling annual growth increments [*Beukema et al.*, 1985; *Tanabe and Oba*, 1988; *Kraeuter and Castagna*, 2001; *Schöne et al.*, 2003; *Fenger et al.*, 2007; *Goewert et al.*, 2007; *Goewert and Surge*, 2008; *Rodland et al.*, 2009, and references therein]. Our findings, however, suggest that the annually formed nacre tongues are independent of temperature. Specimens A16 and B16 formed nacre tongues and the onset of translucent layers during decreasing $\delta^{18}O$ values (i.e., warming months in late to early spring), whereas specimens C30 and D30 secreted them near or at the most positive $\delta^{18}O$ values (i.e., coldest months) (Figure 7). This observation is consistent with delayed water heating below the thermocline (i.e., ~30 m) by advection from warmer surface waters and a fixed timing unrelated to temperature for the formation of the nacre tongues in the four shells.

[33] The corresponding $\delta^{13}C$ values provide additional information on environmental factors that may govern nacre tongue formation. In specimens A16 and B16, carbon and oxygen isotope ratios are in phase with each other; however, they are out of phase in specimens C30 and D30 (Figure 7). The timing of nacre tongue formation with respect to carbon isotope ratios occurs at relatively low values in the sinusoidal time series of all four shells irrespective of depth. Low $\delta^{13}C$ values have been related to high concentrations of chlorophyll a and phytoplankton [*Schöne et al.*, 2005b]. Increasing food availability rather than temperature may control nacre tongue formation in *P. nobilis*. Alternative hypotheses are gonad maturation and reproduction. Gonad

maturation is a possible factor because it starts in spring [*de Gaulejac*, 1995]. Reproduction, however, probably does not influence nacre tongue formation because it occurs only once a year around September in the western Mediterranean [*Kersting and García-March*, 2007; *Cabanellas-Reboredo et al.*, 2009], which does not agree with our isotopic results. The relationship between nacre tongues and oxygen and carbon isotope records may be useful in identifying specimens from different populations. Using the patterns of deposition of shell records and isotopic values it will be possible to reduce the bias derived from using shells from different positions relative to the thermocline. This feature is of great importance in order to create master chronologies from *P. nobilis*.

[34] The combination of isotopic data and growth features enabled us to decipher patterns within individuals that allow us to determine season of death. We used the relationships between the position of translucent layers observed relative to nacre tongues and $\delta^{18}O$ values to infer the date of each animal's death. The relationships between growth features and timing relative to the $\delta^{18}O$ time series is consistent within an individual (Figure 7). For example, in specimen A16, nacre tongues form immediately after the coldest months and translucent layers continue forming until shortly after the warmest months. As stated in section 4, specimens A16, B16 and C30 died some time between late spring 2007 and early spring 2009. At the growing edge of the nacre layer, specimen A16 had formed a complete translucent layer and was starting to form an opaque one, indicating it died in late autumn to early winter. Specimen B16 died before starting to form the translucent layer, just after a nacre tongue had been formed; therefore, it died around mid spring to early summer. Specimen C30 deposited some translucent layers after the last nacre tongue, indicating that the moment of death would be also around mid spring to early summer. Since in late spring 2007 all animals were alive, we estimated that the season of death was around spring to early summer 2008 for specimens B16 and C30. Because specimens A16 and B16 were found dead in living position and the epibiont cover on the inner side of both shells was very similar, we infer that the moment of death for specimen A16 was soon after B16 in autumn–winter 2008. Counting back the nacre tongues from the estimated season of death, we confirmed the periods sampled for isotopic analysis include late spring 2002 to early spring 2005 for specimen A16, from summer 2001 to late spring 2003 for specimen B16, and from spring 1995 to winter 1997 for specimen C30. Specimen D30 had formed a complete translucent layer when it died, indicating death occurred around late summer/early fall. The similarities among the peaks of calcification temperatures of shell D30 with measured temperatures during the period 2005–2007 indicate that this animal died in autumn 2008 and had been dead for around 2 and a half years when it was collected in July 2010.

7. Conclusions

[35] The new methodology for calcite sampling provides a precise record of *P. nobilis* shell $\delta^{18}O$ and $\delta^{13}C$ values with a clear annual sinusoidal pattern, and allows reconstruction of seasonal variability in marine climate with higher reso-

lution (e.g., monthly to submonthly) across a wider range of growth years than attainable in earlier studies of *P. nobilis*.

[36] Oxygen isotope ratios in *P. nobilis* shells above and below the thermocline reflect differences in water temperature and salinity that can be used as environmental geochemical indicators. The carbon isotope ratios of shells C30 and D30, sampled in years 13–15 and 11–13 of the growth period, respectively, are more negative and show lower amplitudes than those of shells A16 and B16, sampled in years 5–7 and 4–6, of the growth period, respectively. Our observations are consistent with the ontogenetic trend toward lower $\delta^{13}C$ values with age found in previous studies of *P. nobilis* [*Kennedy et al.*, 2001; *Freitas et al.*, 2005] and supports the hypothesis that an increased quantity of metabolic carbon is incorporated in the shell with aging in this species.

[37] We found an offset equivalent to ~6.1°C (~1.4‰) between instrumental and calcification temperatures that cannot be explained by incorrect assumptions about our $\delta^{18}O_{WATER}$ value or by growth cessations. The offset also occurs in other *P. nobilis* studied by previous authors, although it had not been reported in those earlier studies. It appears to be consistent through the year and between individuals and locations so it does not restrict the use of $\delta^{18}O$ in *P. nobilis* as a paleoclimate proxy.

[38] The inner layer formed by a nacre tongue and a translucent and opaque layer is deposited annually and its periodicity is unchanged with ontogeny. The number of nacre tongues can be used to age animals. Neither nacre tongues, nor translucent and opaque layers are related to water temperature, but to an event occurring in spring. Food availability or gonad development are the most probable causes for their formation. The relationship between nacre tongues and oxygen and carbon curves could be used to identify specimens from different populations. This feature is of great importance in order to create master chronologies from *P. nobilis*.

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