Age, growth rate and season of recruitment of *Pinna nobilis* (L) in the Croatian Adriatic determined from Mg:Ca and Sr:Ca shell profiles

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Abstract

Stable oxygen isotope analyses of U-shaped spines removed at intervals along profiles of the outer shell surface of *Pinna nobilis* (L) were used to reconstruct sea surface temperature (SST) and validate the periodicity of adductor muscle scar rings on the inner shell surface. Elemental ratios (Mg:Ca and Sr:Ca) of spines, determined using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), were compared with the SST estimated from the stable oxygen isotopic composition of the shell deposited at the same time. The elemental ratios and the stable oxygen isotopic composition recorded in the shell were significantly correlated: Mg:Ca ratio = 0.0002 (seawater temperature) + 0.0095 (r^2 = 0.445), Sr:Ca ratio = 0.00005 (seawater temperature) + 0.0014 (r^2 = 0.887). The ratios in the spines were highest when the SST was warmest during July and August and were lowest between January and February when the SST was minimal.

The positions of the first and second adductor muscle scar rings, unlike the later rings, are often difficult to discern, and in large shells they are obscured by nacre. Seasonal patterns in the elemental ratios were used to characterise the age in regions of the outer shell surface corresponding to the first two years of shell growth. A combination of both elemental ratios and muscle scar rings were used to estimate the age and hence the growth rate of *P. nobilis* from three locations in the Croatian Adriatic. Annual cycles of shell growth, inferred from the seasonal pattern in the element ratios, were used to determine the season of recruitment of fan mussels at several localities along the Croatian coastline. Pinnids generally settled during late autumn and winter although one shell from Mali Ston Bay settled during the summer. *P. nobilis* from Mali Ston Bay exhibited the fastest growth reaching a

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length of ∼ 60 cm and an age of 9 years, whereas those from Malo jezero grew the slowest attaining a length of ∼ 50 cm at 12 years of age.

1. Introduction

Fan mussels *Pinna nobilis* are large (size 60–120 cm) and impressive bivalves (Zavodnik et al., 1991; Richardson et al., 1999) which live partially buried by the anterior portion of the shell and attached by their bysuss in the substratum amongst sea grass *Posidonia oceanica* (Delile) and *Cymodocea nodosa* (Ucria) meadows. They used to be common inhabitants of Mediterranean coastal waters, but over the last few decades their numbers have declined. Their demise can be attributed to the reduction and loss of their natural habitat such as sea grass meadows, to an increase in anthropogenic inputs into coastal waters resulting from the development of holiday resorts and to the collection of shells by amateur divers (Vicente, 1990; Zavodnik et al., 1991; Vincente and de Gaulejac, 1993). As in many parts of the Mediterranean, *P. nobilis* used to be a common inhabitant of the shallow coastal waters of the Adriatic along the coastline of Croatia (Zavodnik, 1967). However, little research has been conducted in this region into temporal changes in population structure, reproduction and recruitment success of *P. nobilis* (Zavodnik, 1967; Zavodnik et al., 1991; Šiletić and Peharda, 2003). In parts of the Mediterranean, *P. nobilis* is a protected species and many coastal waters have been designated as marine parks (e.g. Mljet Island National Park, Croatia and Parque Natural de Cabo de Gata-Nijar, Spain). Fan mussels are usually patchily distributed and rare making them difficult organisms to study routinely.

Informed decisions regarding the protection of *P. nobilis* depend on an ability to estimate accurately the density of fan mussels within a given area and the age and growth rate of individuals within the populations. Several methods have previously been used to study the growth of *P. nobilis* including (1) the in situ marking and measurement of tagged individuals (Hignette, 1983; De Gaulejac and Vicente, 1990; Butler et al., 1993; Šiletić and Peharda, 2003), (2) counting and measuring the distance between the annual adductor muscle scars on the inner shell surface (Richardson et al., 1999), and (3) determination of seasonal changes in sea surface temperatures and hence the inference of seasonal growth rates from stable oxygen isotopes taken from transects along the growth axis of the shell surface (Richardson et al., 1999; Kennedy et al., 2001). These methods have their advantages and disadvantages. The advantage of making repeated in situ measurements of fan mussels is that a large number of individuals can be processed and observed without the necessity of removing them and sacrificing them for study, a method which is particularly valuable in marine conservation areas where it is prohibited to remove *P. nobilis*. However, since fan mussels live partially buried in the sediment, they have to be disturbed when the anterior portion of the shell is located during measurement and this may destabilise their hold in the sediment and lead to disturbances in shell deposition.

Data collected from these kinds of study usually show a high degree of variability and in slow-growing specimens, where the annual increment of growth is small, it is often difficult to repeat measurements along the same axis (Šiletić and Peharda, 2003). Determination of the age of pinnids from the internal adductor muscle scar rings requires the sacrifice of a number of individuals (Richardson et al., 1999). Also, identification of the first and second annual scar rings can be problematical owing to the deposition of nacre in later life which obscures the first few underlying annual growth scars. Generally, the data generated from measurements of the growth scars are less variable than those obtained by in situ measurements of individual fan mussels. A problem associated with the determination of seasonal seawater temperatures using stable oxygen isotopes is that it is often difficult to obtain the required spatial resolution of samples, particularly in fan mussels older than four years of age where the annual growth increment is small (Kennedy et al., 2001). The methodology of drilling small samples at discrete intervals along the growth axis of the shell can be time consuming and can be expensive to analyse all but a few shells using this technique.

The outer shell surface of *P. nobilis* is ideally suited for the analysis of the elemental composition of the calcium carbonate shell since it is adorned across much of its surface with small (1–5 mm long) U-shaped calcite spines. Around the margin of the shell, there may be as many as 40 spines depending on the size of the shell. These spines can quickly and easily be removed by fine forceps for stable isotopic (Richardson et al., 1999; Kennedy et al., 2001) and elemental analyses. In this paper we: (1) determined the stable isotopic composition, as a proxy for sea surface temperature (SST), of spines removed at regular intervals along a transect across the shell surface of a *P. nobilis* shell, (2) determined the Mg:Ca and Sr:Ca ratios in calcite spines and drilled calcite samples taken from profiles along the outer shell surface of six *P. nobilis* collected from several localities along the Croatian coastline. This enabled us to characterise the age and growth rate of these pinids in regions of the shell corresponding to ages < 3 years where the adductor muscle scar rings were not discernible in the first formed regions of the shell. Since trace element uptake in calcite and aragonite bivalve shells has been shown to follow regular patterns with respect to environmental variables such as temperature, salinity, growth rate and nutrient supply (Dodd and Crisp, 1982; Fuge et al., 1993; Stecher et al., 1996; Klein et al., 1996a,b; Richardson, 2001), seasonal patterns of shell growth and hence age were inferred from the elemental data, (3) we compared the isotopically derived SST with the Mg:Ca and Sr:Ca ratios in the spines to establish a relationship between SST and elemental composition. This information was then used to predict the season of recruitment of the fan mussels and (4) we used a combination of adductor muscle scar rings and seasonal patterns in elemental ratios to age the *P. nobilis* shells and determine their annual shell growth rates.

2. Materials and methods

P. nobilis were collected at three sites from the coastal waters of Croatia in the south east Adriatic Sea (Fig. 1). Malo jezero ('Little lake', site 1) and Veliko jezero ('Big lake', site 2) are protected marine lakes and form part of the Mljet Island National Park. Malo
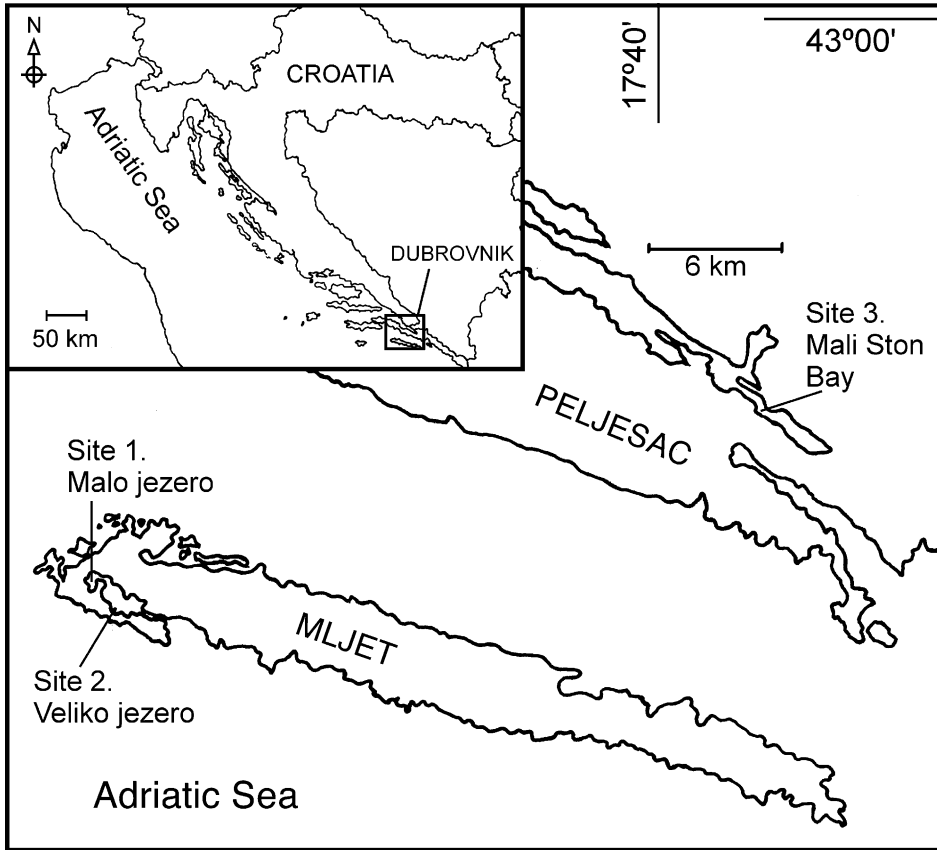
Fig. 1. Location of the study sites in the Adriatic Sea. Site 1 (Malo jezero), site 2 (Veliko jezero) and site 3 (Mali Ston Bay).

jezero became a salt water lake (previously it had been a freshwater lake) about 5000 years ago (Wunsam et al., 1999) and is connected to Veliko jezero through a shallow and narrow channel, which is in turn connected to the surrounding open sea through a somewhat deeper and wider channel (Vuletić, 1953). Both lakes are characterized by the input of run-off from the surrounding terrestrial area and by restricted communication with the open sea (Benović et al., 2000). Mali Ston Bay (site 3), is a protected marine park with a long tradition of European flat oyster (*Ostrea edulis* Linnaeus 1758) and black mussel (*Mytilus galloprovincialis* Lamarck 1819) aquaculture and is characterized by strong currents and freshwater springs. Owing to the protected status of the sampling localities, and endangered species status of *P. nobilis* in Croatia, it was only possible to obtain permission to remove 18 fan mussels for study. Six specimens of *P. nobilis* (size range 15–62 cm) were collected by SCUBA divers from each of the three sites, from site 1 (Malo jezero) and site 2 (Veliko jezero) in December 2000 and from site 3 (Mali Ston Bay) in February 2001.

It has previously been established that the adductor muscle scars on the inner shell surface of *P. nobilis* from Spanish waters (see Fig. 2) are laid down annually (Richardson et al., 1999). Counting these scars estimated their age but not their size at a particular age. To relate the position of each scar to the outer surface of the shell, a relationship between the length of the adductor muscle scar and shell length was established (Richardson et al., 1999). Thus any linear measurement on the outer shell surface could be related directly to
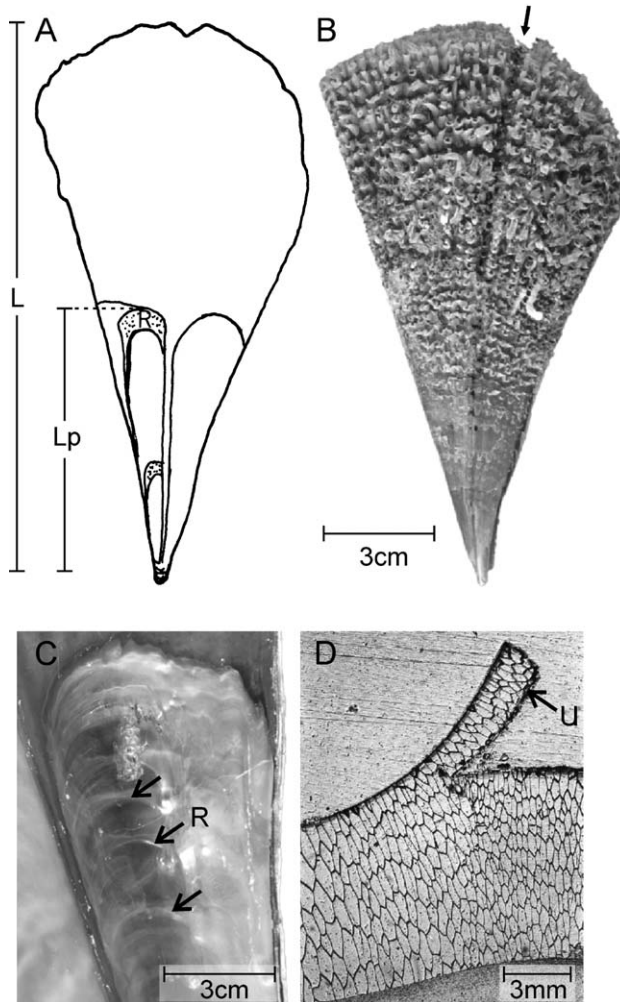

Fig. 2. *P. nobilis*. (A) Diagrammatic appearance of the inner shell surface to illustrate the position of a ring (R) on the internal posterior adductor muscle scar. *L*: shell length; Lp: length of the posterior adductor muscle scar. (B) Photograph of the external shell surface adorned with small spines. Arrow indicates the transect along which the pairs of spines were removed for analysis. (C) Photograph of the posterior adductor muscle scar showing several clear rings (R) (arrows). (D) Photomicrograph of a section through the outer shell layer and a spine (U) to show the prismatic shell structure is contiguous through both. Scale bars in B and C = 3 cm and in D = 3 mm.

a measurement on the adductor muscle scar and vice versa. A similar relationship was established in this study. Shell length (L) and total length of the posterior adductor muscle scar (Lp) (Fig. 2) of the 18 Croatian pinnids were measured to the nearest 0.1 cm using vernier callipers and the relationship between shell length (L) and length of the posterior adductor muscle scar (Lp) found. The relationship can be explained by the following equation:

$$L = 1.1 + 2.36 \text{Lp} \qquad (n = 18, \ r^2 = 0.989) \tag{1}$$

Using this relationship, a (~ 12 cm) length of the outer shell surface of one *P. nobilis* (shell 1, $L = 36.5$ cm) from Malo jezero was selected which corresponded to the distance between the first and second putative annual adductor muscle scar rings and defined a year of growth and an annual SST cycle. A pair of adjacent U shaped spines (each $\sim 2–3$ mg in weight) and free of fouling epibiota (e.g. bryozoans and serpulid tube worms) was removed at ~ 0.5 cm intervals using fine forceps from the outer surface along a transect down the mid-axis of the shell (Fig. 2) (a total of 24 pairs of spines); one spine was analysed for its stable oxygen isotopic composition (δ^{18}O) and the other spine was subject to elemental analysis (Ca, Mg and Sr). We have previously established that individual spines deposited at the same time around the margin of the shell of *P. nobilis* have a similar stable isotopic composition (unpublished observation). The SST at the time of shell deposition, determined from the δ^{18}O in one of the spines, was compared with the Mg:Ca and Sr:Ca ratios in the replicate spine to ascertain whether the element ratios displayed a seasonal pattern of formation and whether they could be used as a proxy for SST.

For stable oxygen isotope analysis, each one of the pair of spines was homogenised separately and isotopic analyses performed automatically using a PDZ-EUROPA 20/20 mass spectrometer with automatic carbonate system (CAPS) (see Kennedy et al., 2001). Values are reported in per mil notation relative to PDB. The overall analytical precision of the internal standard for δ^{18}O of the calcite samples was 1.53 ($n = 8$, $1\sigma = 0.02\%$). A relationship between oxygen-isotope composition of seawater (δ_W), shell carbonate (δ_c) and temperature has been defined by a paleotemperature equation formulated by Epstein et al. (1953) and later modified by O'Neil et al. (1969), (Eq. (2)):

$$T°C = 16.9 - 4.38(\delta_\text{c} - \delta_\text{W}) + 0.01(\delta_\text{c} - \delta_\text{W})^2.$$

A value of 1.83‰ for δ_W was determined from seawater samples collected in December from Malo jezero at a similar depth (5 m) to that which the *P. nobilis* were collected. Salinity measured at this depth varied by 1.3 psu (Benović et al., 2000) and resulted in a maximum variation in the estimated seawater temperature of ~ 2 °C.

For the analysis of Mg:Ca and Sr:Ca ratios, each one of the other pair of replicate whole spines ($\sim 2–3$ mg) was dissolved separately in 2 ml of nitric acid and made up to a 25 ml solution with double distilled water. Samples were analysed on a JY138 Ultrace Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES). The analytical precision for Ca was better than 4% and better than 5% for Mg and Sr.

Five small pinnids (15–36 cm) (shells 2–6) from the 18 shells collected either did not have a clear first and second adductor muscle scar ring or the precise position of these rings was ambiguous. Based on the relationship between δ^{18}O and the Mg:Ca and Sr:Ca

ratios determined above, seasonal variations in these ratios along a transect running down the mid-axis of the length of shell 1 and of the five shells (2–6) were determined. One shell from Malo jezero (shell 2) ($L = 15.0$ cm), two shells from Veliko jezero (shells 3 ($L = 27.1$ cm) and 4 ($L = 15.5$ cm)) and two shells from Mali Ston Bay (shells 5 ($L = 26.0$ cm) and 6 ($L = 28.8$ cm)) were selected. Whilst the entire length of shells 1–5 was sampled, only the first 10 cm was analysed in shell 6. Since shells 5 and 6 were of similar size we were primarily interested in determining the season of settlement and the position of the first adductor muscle scar ring in shell 6, and this did not require the complete analysis of the shell. U-shaped spines were removed at ~ 0.5 cm intervals as described previously. However, near the apex in the oldest part of the shell, the spines were abraded and absent, a distance of < 7 cm. Here discrete samples of calcium carbonate were drilled at ~ 0.5 cm intervals along the transect down the mid-axis of the shell using a 0.6 mm dentist drill. Great care was taken to ensure that only the outer calcite layer was abraded and that the drill did not penetrate into the underlying aragonite nacre layer. If there was any doubt that sample integrity had been compromised, then these samples were not analysed. In order to check that the spines and outer shell layer had a similar structure, small areas of shell (~ 4 cm^{-2}), with attached spines were embedded in resin and acetate peel replicas of polished and etched shell and spine sections prepared (Richardson, 2001) The outer shell layer and spines are structurally the same (see Fig. 2D) and are therefore assumed to have a similar elemental composition. Each drilled sample was an homogeneous sample of calcium carbonate whereas the spine was a discrete structure. Elemental analyses (Mg:Ca and Sr:Ca ratios) were undertaken on drilled homogeneous samples from the older regions of the shell and on spines from the younger part of the shell; similar weights of spine and homogeneous samples (~ 2–3 mg) were analysed. From these data, the number of annual cycles in the Mg:Ca and Sr:Ca ratios present in the shells and the approximate season of spat settlement were determined.

The clearly defined adductor muscle scars present on the inner surfaces of the 12 *P. nobilis* shells were used to age each shell and the distance from the shell apex to the centre of each adductor muscle scar ring (Lr) measured to the nearest 0.1 cm (see Richardson et al., 1999). Shell length at each ring was estimated using Eq. (1). In the other six shells whose age had been established from the seasonal cycles in the Mg:Ca and Sr:Ca ratios, the distance between the shell apex and the position on the shell of each lowest element ratio (equivalent to the winter, December to April period) similarly measured. Length age data were fitted to the von Bertalanffy growth function $Lt = L_{\alpha}(1 - e^{-k(t-t0)})$ using the Fisheries Programme "Fishparm".

3. Results

No obvious growth rings were present on the surface of the fan mussel shells from the three sites although two shells (2 and 4), displayed check marks resulting from physical damage. Clear concentric rings were present on the inner shell-surface of the posterior adductor muscle scars of the large *P. nobilis*, although they were not readily obvious in the small (< 25 mm) pinnid shells. In the largest shells, there was the possibility that the earliest deposited muscle scars had become obscured by nacre which is deposited on the

inner surface of the pinnid shell to thicken the shell as it grows in length. At the margin of the adductor muscle scars of the large shells collected in December, there was evidence of the initiation of ring formation, and by February, a ring was discernible although deposition was not complete. Seawater temperature varies seasonally at Malo jezero (Fig. 3A) with maximum (29 °C) and minimum (10.5 °C) temperatures recorded in July/
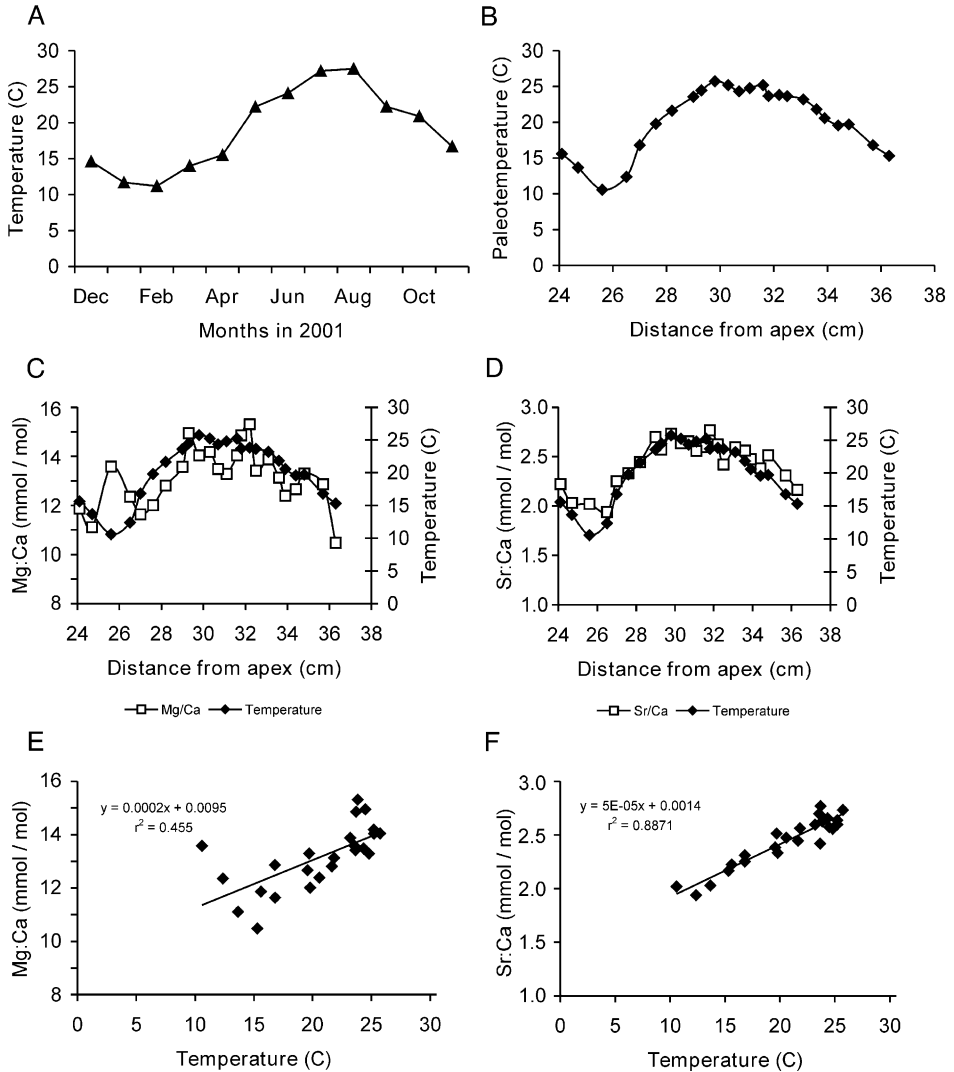

Fig. 3. (A) Seasonal variation (2000–2001) in sea surface temperature (SST) at Malo jezero, Croatia; (B–F) Data from *P. nobilis* from Malo jezero, Croatia. (B) Shell 1 from Malo jezero, inferred SST profile calculated from the stable oxygen-isotope composition in surface spines from a section of the outer surface; (C, D) comparison of Mg:Ca and Sr:Ca ratios, respectively, and inferred SST from surface spines from the same section of shell in B; (E, F) linear relationship between Mg:Ca and Sr:Ca ratios, respectively, and inferred SST.

August and February/March, respectively. The appearance of the adductor muscle scar rings between December and February suggests they were formed when seawater temperatures were minimal in the winter and early spring.

The palaeotemperature equation was used to predict the SST at the time of deposition of the spines on shell 1 (Fig. 3B). Two periods of low SST (10 and 15.5 °C) were recorded respectively in the spines removed from the shell at 25.9 cm and at the shell margin (36.5 cm), when the animal died after collection during December. These temperature minima are located close to the extrapolated positions (estimated using Eq. (1)), of the adductor
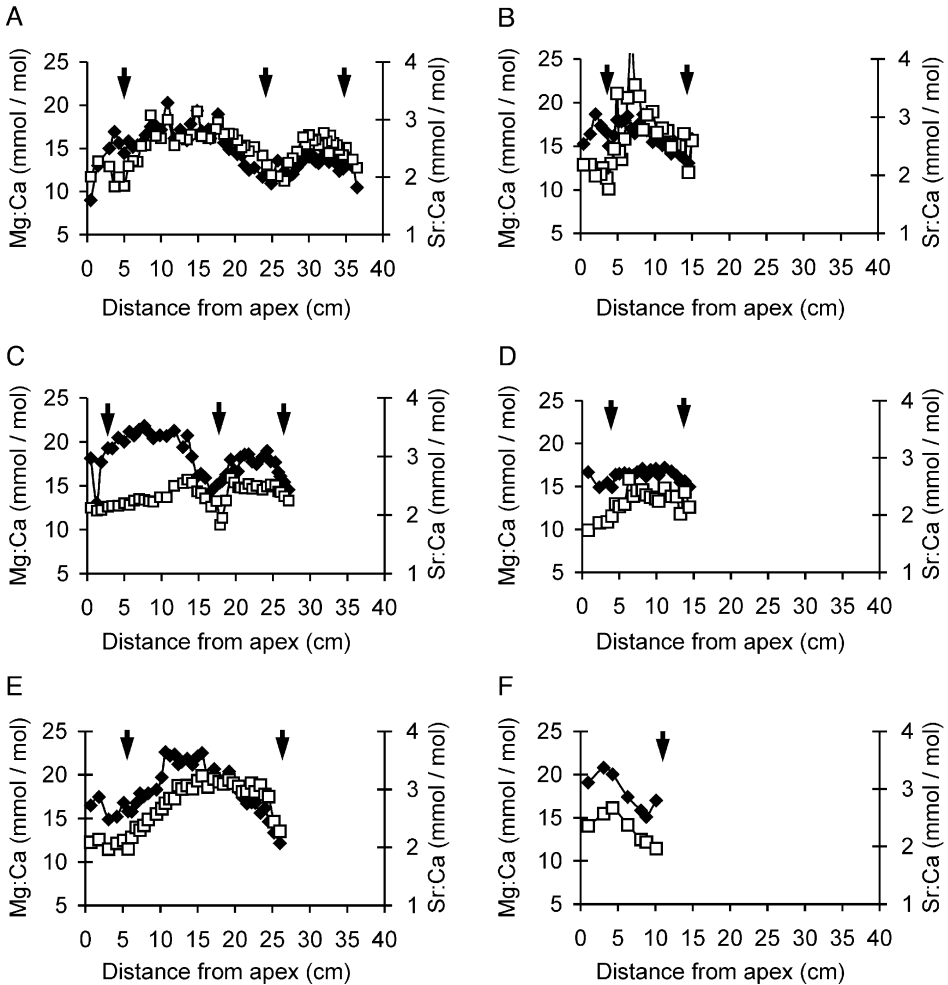

Fig. 4. Trace element Mg:Ca (♦) and Sr:Ca (□) ratio trends in surface spines and drilled shell samples for shells 1–6. (A, B) Shells 1 and 2 from Malo jezero (length 36.5 and 15.0 cm, respectively), (C, D) shells 3 and 4 from Veliko jezero (length 27.1 and 15.5 cm, respectively), and (E, F) shells 5 and 6 from Mali Ston Bay (length 26.0 and 28.8 cm, respectively). Only the first 12 cm of shell from the apex was analysed in shell 6. Arrows indicate the positions of the Mg:Ca minima and periods of winter shell deposition.
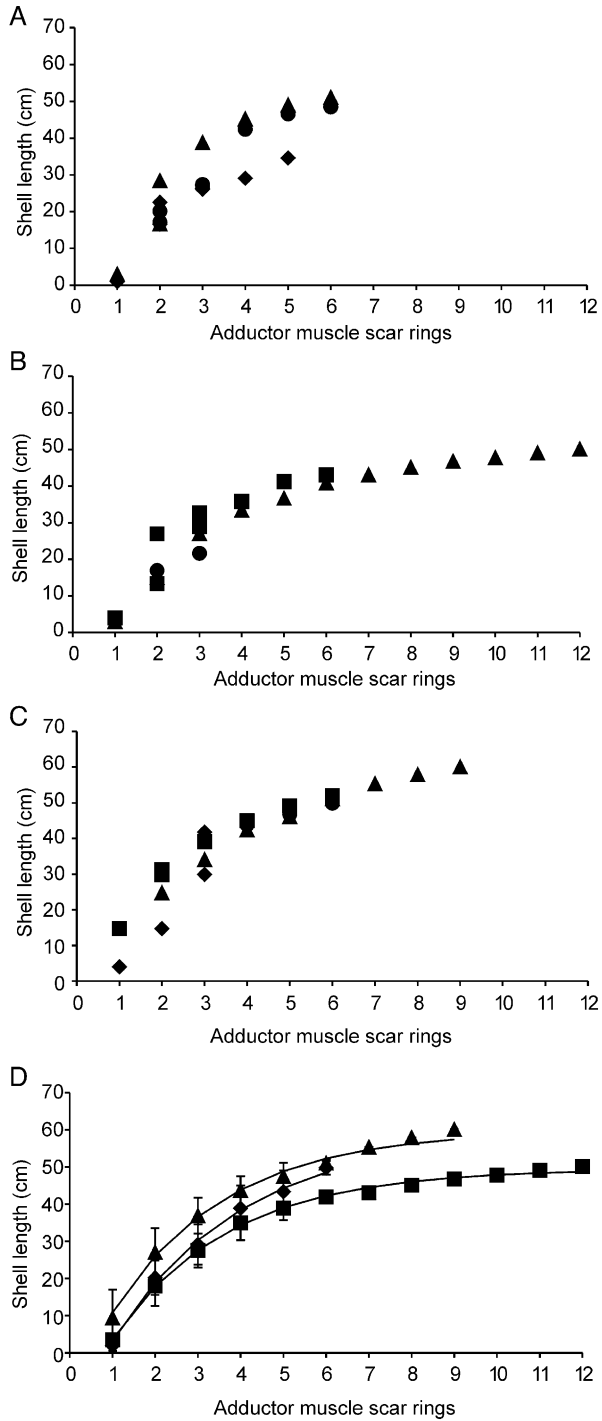
muscle scar rings on the shell surface. Fig. 3C and D compares the spine Mg:Ca and Sr:Ca ratios with the $\delta^{18}O$ derived SST record. With the exception of an anomalously high Mg:Ca and Sr:Ca ratio at 25.9 cm, the highest ratios coincided with maximum seawater temperatures during the summer (July and August), whereas the lowest ratios occurred during the winter (February to March) when seawater temperatures were at their lowest. Both ratios were significantly correlated with seawater temperature; Mg:Ca ratio = 0.0002 (seawater temperature) + 0.0095 ($r^2 = 0.445$) ($P < 0.001$), Sr:Ca = 0.00005(seawater temperature) + 0.0014 ($r^2 = 0.887$) ($P < 0.001$) (Fig. 3E and F).

Fig. 4(A–F) shows the variation in the Mg:Ca and Sr:Ca ratios from the apex to the shell margin in shell 1 (included are additional elemental data to that shown in Fig. 3C and D), and in the five small shells (2–6) from the three localities. The last spine removed from the shells was taken at the time of death during the winter, either in December or February. Cycles of maximum and minimum (arrows) ratios are apparent. For the Mg:Ca ratios, shells 1 and 2 from Malo jezero displayed 3 and 2 ratio minima, respectively, shells 3 and 4 from Veliko jezero, displayed 3 and 2 ratio minima, respectively, and shells 5 and 6 from Mali Ston Bay displayed 2 and 1 ratio minima, respectively. Generally, the Sr:Ca ratios followed the seasonal pattern in the Mg:Ca ratios, although shell 3 was anomalous in that the Sr:Ca ratios did not track the Mg:Ca ratios over the first 10 cm of shell.

The season of settlement of each shell was estimated from a comparison of the trends between the elemental ratios and the seawater temperature. The trend in elemental ratios at the apex in shells (1 to 5) is consistent with a SST during December of ∼ 12 °C. The variation in the ratios over the first 10 cm in shell 6 collected in February from Mali Ston Bay was anomalous with a seasonal pattern more consistent with a settlement date during late summer or early autumn. The overall pattern in this shell was similar to the seasonal pattern displayed at the shell margin of shell 5, also collected in February from Mali Ston Bay; the ratios at the apex in this shell, however, suggested a late autumn settlement rather than a summer settlement. Using the relationship between the elemental ratios and SST, we estimated that the check marks, probably arising from physical damage, observed on Pinna 2 from Malo jezero at a length of between 8.6 and 9.6 cm occurred during the summer and on Pinna 4 from Veliko jezero at a length of between 10.7 and 11.2 cm occurred during the late summer/early autumn.

The size of the shell at the point where the element ratios were minimal in the winter were substituted, together with measurements of the size at age determined from the clearly discernible adductor muscle scar rings, into the Von Bertalanffy growth equation and growth curves fitted to the size at age data for fan mussels from each site (Fig. 5). Most of the shells studied were < 6 years old although two of the largest pinnids from Mali Ston Bay and Malo jezero reached an age of 9 and 12 years old, respectively. Considerable variability in the size at the first and second annual rings was noted in all the shells (Fig. 5A–C). Whilst the number of shells analysed (6 from each site) are too small for

Fig. 5. P. nobilis. (A–C) Size at age data for the six pinnids from (A) Veliko jezero, (B), Malo jezero and (C) Mali Ston Bay (▲). (D) Curves for Veliko jezero (◆), Malo jezero (■) and Mali Ston Bay (▲) fitted to the mean (± S.D., lower and upper limits only shown) using the Von Bertalanffy growth equation: Veliko jezero, $L = 59.3$ $(1 - e^{-0.331t - 0.831})$; Malo jezero, $L = 49.5$ $(1 - e^{-0.369t - 0.788})$; Mali Ston Bay, $L = 59.9$ $(1 - e^{-3.806t - 0.463})$.

significance, there is a suggestion that some pinnids reach a length of between 13 and 22 cm at the formation of the second muscle scar, whilst others from the same population attain a size of 25–31 cm at this same point in time. From the elemental analyses of six of the shells, five shells appeared to deposit the first muscle scar at a size of between 1 and 4 cm, whereas one shell from Mali Ston Bay (no.6) formed the first ring at 14.7 cm. This pinnid is considered to have settled during the summer whilst the others settled in the late autumn and winter. If there were two settlement periods, then this could account for the difference in lengths of the pinnids at the first and second muscle scar rings, observations which are supported by the elemental analyses.

4. Discussion

The age of bivalves is conventionally determined using annual surface growth-rings, checks or internal growth lines (Richardson, 2001). Clear external growth annuli are generally absent from fan mussel shells; however, rings are obvious on the internal surface of the adductor muscle scars on the inner surface of the shell (Richardson et al., 1999). In the largest specimens examined (>25 cm), there was evidence of the initiation of ring formation on the adductor muscle scar of shells collected in December from Malo jezero and Veliko jezero, and by February, a ring was clearly discernible at the growing edge of the muscle scar in shells collected from Mali Ston Bay, although deposition was not complete. This suggests ring deposition occurs during the winter and is completed by the early spring when seawater temperatures are increasing. Richardson et al. (1999) demonstrated from stable isotope records in the shell of *P. nobilis* from S.E. Spain that adductor muscle scar rings were formed when seawater temperatures were increasing from 14 °C in early summer. Like the Spanish pinnids, the adductor muscle scar rings on the Croatian fan mussels represent an annual event. One obstacle to determining the age of all the sizes of the Croatian *P. nobilis* collected in this study was the apparent lack of any clearly discernible adductor muscle scar rings on the smaller shells (<25 cm), a problem that has been encountered elsewhere in studying pinnids (Richardson et al., 1999) and in other bivalves (Richardson, 2001).

Another difficulty that can arise in estimating the age of *P. nobilis* is that the first ring can be obscured by later deposition of nacre on the inner surface of the adductor muscle scar. In some cases, the absence of a first ring can be compensated for when estimating a pinnid's age. Richardson et al. (1999) determined the stable isotopic composition along transects of the outer surface of the shell of *P. nobilis* from the Spanish Mediterranean in which there was doubt about the position of the first ring on the adductor muscle scar. From the estimated seawater temperatures, cycles of shell growth were inferred and it was observed that shells of ∼25 cm displaying only one clear adductor muscle scar ring were actually 2 years of age; the first ring was obscured by nacre deposited on the internal shell surface. Growth curves that were subsequently generated from these data accounted for the absence of the first ring. In the present study we chose a different approach. We investigated the seasonal variation in Mg:Ca and Sr:Ca ratios in the spines as a proxy for SST and hence seasonal cycles of shell growth. A significant correlation was found between these elemental ratios and the stable oxygen isotopes recorded in the shell; the

highest Mg:Ca and Sr:Ca ratios were present in the spines when the SST was warmest during July and August and the ratios were lowest when the SST was minimal. When we examined the elemental ratios in the spines from a range of different sized pinnids from the three localities, although we found temperature-related cycles of incorporation, there was variation between individual shells and between shell 1 in which we compared the elemental ratios and the SST determined from the stable oxygen isotopes. Therefore the determination of SST's directly from the Mg:Ca and Sr:Ca ratios in the spines was not possible.

There is considerable debate about the environmental significance of Mg:Ca and Sr:Ca ratios in mollusc shells (Rosenberg and Hughes, 1991; Klein et al., 1996b; Putten et al., 2000). Until recently, it was generally accepted that there was a correlation between Mg:Ca ratios in the shells of calcitic bivalves and ambient seawater temperature (Dodd and Crisp, 1982; Klein et al., 1996b); the largest ratios coincided with the warmest seawater temperatures. These ratios in coral aragonite are frequently used as a geothermometer (Hart and Cohen, 1996; Mitsuguchi et al., 1996). Putten et al. (2000), however, have shown that skeletal Mg in *Mytilus edulis* covaries only with temperature during the spring and that this covariation is abruptly interrupted after the spring phytoplankton bloom, thus whilst seawater temperatures continue to increase during the summer, Mg:Ca ratios decrease. Putten et al. (2000) observations suggest internal control of trace element incorporation into the calcite shell may, under certain growth conditions, be independent of environmental conditions. Dodd (1965) demonstrated seasonal variation in the strontium concentration of both the calcite and aragonite in *M. edulis* shells and reported a small positive correlation between strontium and seawater temperature in the outer calcitic prismatic shell layer, whereas strontium was inversely related to temperature in the aragonite nacreous layer. Seasonal changes in strontium concentrations have been noted in the shells of both living and fossil bivalve shells (e.g. Palacios et al., 1994; Stecher et al., 1996). Stecher et al. (1996) noted that strontium decreased in the shell of a Pleistocene *Mercenaria mercenaria* during the winter growth period but in a living *Spisula solidissima* the reverse occurred, with lower levels incorporated into the shell during the summer growth period. Ontogenetic changes in strontium concentrations have also been observed in the shell of *Mya arenaria*; Sr:Ca correlated positively with increasing age of the shell (Palacios et al., 1994). Conversely large *M. edulis* shells were found to have a slightly lower strontium concentration than small shells at a given temperature (Dodd, 1965).

The present study suggests a positive correlation between SST and magnesium and strontium ratios in the spines of *P. nobilis*; as there was variation in the ratios between individual shells, it was not possible to estimate the SST directly from the elemental records. Using the seasonal variation in the element ratios across selected shells, the position of the first growth cessation was estimated. Some variability in length at the first winter was observed and these differences may be accounted for by variations in the timing of spat settlement. Using the elemental ratios, it was possible to assign an approximate season of settlement, either the late summer/early autumn or late autumn/ early winter. Although only six shells were analysed, we considered these to be representative of the sample of the 18 shells collected. Anecdotal evidence to support an autumn/winter settlement comes from divers, who in December 2001, observed a small pinnid of 3 cm settled in shallow water on ropes used for flat oyster aquaculture. This

independent evidence lends support to our observations from the elemental analyses of a size of 1–4 cm at the first muscle scar ring.

The outer surfaces of the shells of the Pectinidae (e.g. the scallops *Pecten maximus* and *Aequipecten irregularis*) have rows of small striae forming concentric rings across the shell (Owen et al., 2002). The striae are an extension of the outer calcite shell layer and the distance between the striae varies seasonally in a predictable way; inter-striae distance is greatest when seawater temperatures are warm during the summer and narrowest during the colder winter months. In a similar fashion to that demonstrated with *P. nobilis* in this study, individual striae could be removed from the scallop shell for elemental analyses to answer questions such as the role of temperature in controlling shell chemistry. Bivalve and gastropod shells are amenable for element analyses and can be sampled by drilling the outer surface or by sampling specific areas of the sectioned shell. The shells also contain a chronology of their shell growth in the form of annual growth lines and there is great potential for investigating historical changes in element chemistry, e.g. anthropogenic inputs and upwelling events (for review see Richardson, 2001).

Pinnids from Mali Ston Bay exhibited the fastest growth reaching a length of ~ 60 cm and an age of 9 years, whereas those from Malo jezero grew the slowest, were older (12 years) and attained a length of ~ 50 cm. Malo jezero is the smaller of the two lakes in the Mljet Island national Park and is connected to the larger marine lake Veliko jezero through a small and narrow channel. The channel into Malo jezero may restrict the supply of food, and modify the environmental conditions in the marine lake and the supply of potential pinnid spat, factors which may contribute to slower growth rates of pinnids in this location. The growth rates attained by some of the Croatian pinnids are similar (50 cm in 12 years) to those achieved by pinnids from the Spanish Mediterranean sites studied by Richardson et al. (1999). Spanish pinnids from Aguamarga reached a length of ~ 45 cm in 12 years, whereas those from Carboneras attained a size of ~ 55 cm when they were 7 years of age.

The present study has demonstrated the potential use of trace elements in the shell of *P. nobilis* for resolving questions regarding the age of fan mussels in Croatian waters and for estimating the season of spat settlement. *P. nobilis* is vulnerable to exploitation and although there are encouraging signs that the coastal waters of the Mediterranean are achieving protected status, there is evidence in the Mljet national park that pinnids are still being removed. A recent diving excursion during July 2001 revealed the presence of ~ 20 large (>40 cm) pinnid shells discarded on the seabed (Peharda, personal observation). Through an understanding of the natural patterns of recruitment and growth of *P. nobilis*, suitable management strategies could be implemented to regulate and help control the removal of piniids.

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