Growth-increment characteristics and isotopic ($\delta^{18}O$) temperature record of sub-thermocline *Aequipecten opercularis* (Mollusca:Bivalvia): evidence from modern Adriatic forms and an application to early Pliocene examples from eastern England

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ABSTRACT

The shell δ^{18}O of young modern *Aequipecten opercularis* from the southern North Sea provides an essentially faithful record of seasonal variation in seafloor temperature. In this well-mixed setting, *A. opercularis* shell δ^{18}O also serves as a proxy for seasonal variation in surface temperature. Individuals from less agitated (e.g. deeper) settings in a warm climate would not be expected to record the full seasonal range in surface temperature because of thermal stratification in summer. Such circumstances have been invoked to explain cool isotopic summer temperatures from early Pliocene *A. opercularis* of eastern England. Support for a sub-thermocline setting derives from high-amplitude variation in microgrowth-increment size, which resembles the pattern in sub-thermocline *A. opercularis* from the southern Mediterranean Sea. Here, we present isotope and increment profiles from further sub-thermocline individuals, live-collected from a location in the Adriatic Sea for which we provide modelled values of expected shell δ^{18}O. We also present data from supra-thermocline shells from the English Channel and French Mediterranean coast. The great majority of sub-thermocline *A. opercularis* show high-amplitude variation in increment size, and winter and summer δ^{18}O values are generally quite close to expectation. However, the relatively warm summer conditions of 2015 are not recorded, in most cases due to a break in growth, perhaps caused by hypoxia. The supra-thermocline shells show subdued increment variation and yield isotopic winter and summer temperatures quite close to the local directly measured values. *A. opercularis* shells therefore provide a fairly good isotopic record of ambient temperature (if not always of relatively warm summer conditions below the thermocline) and their hydrographic setting can be determined from increment data. Early Pliocene examples from eastern England can be interpreted as having lived in a setting below the thermocline, with a higher seasonal range in surface temperature than now in the adjacent southern North Sea.

1. Introduction

The $\delta^{18}O$ of skeletal $CaCO_3$ (calcite and aragonite) is very widely used as a proxy for the temperature of the ambient environment; in particular, the $\delta^{18}O$ of marine mollusc shells is used as an indicator of seawater temperature (e.g. Schöne and Surge, 2005; Schöne and Gillikin, 2013; Prendergast et al., 2017; Gillikin et al., 2109). The values obtained are often presented as if they reflect sea-surface temperature, a datum of great interest to climatologists and palaeoclimatologists, but those derived from benthic taxa such as bivalves are of course a record of seafloor temperature (with the additional influence of water $\delta^{18}O$, which must be measured or estimated to enable calculation of temperature from shell $\delta^{18}O$). In agitated settings (where the seafloor is above the fair-weather wave-base, or to somewhat greater depths where tidal currents are strong) stirring of the water is usually sufficient for seafloor temperature to be very similar to surface temperature (Fig. 1A). However, in quieter settings (e.g. at depths below the fair-weather wave-base, in situations where tidal currents are weak), and at latitudes where solar irradiation is significant (i.e. outside the polar regions), seafloor temperature often departs radically from surface temperature in summer (Fig. 1B). This is because the lack of agitation and the lower density of warmed water allows heat to become 'ponded' in a shallow surface layer (commonly extending to 25–30 m in weakly tidal shelf settings), separated from much cooler, deeper waters by a zone of steep temperature change, the thermocline. In the mid-latitudes, where there is significant seasonal variation in insolation, this stratification typically breaks down in autumn because of surface cooling, often aided by storm activity, beginning an equalisation of temperature (Fig. 1B). By the winter months, seafloor temperature is usually about the same as at the surface, to at least mid-shelf depths (e.g. Arthur et al., 1983; Elliott and Li, 1991). Shelf bivalves living below the summer thermocline ('sub-thermocline' individuals) therefore in many cases experience temperatures that are similar to those at the surface in winter (the coldest temperatures occurring at the same time: February/March in the Northern Hemisphere) but experience temperatures that are cooler than those at the surface in summer (the peak temperature at the surface typically occurring during August/September and the lower peak temperature on the seafloor during October/November in the Northern Hemisphere). Sequential sampling of sub-thermocline shells at high temporal resolution through ontogeny (isotope sclerochronology) will provide δ^{18}O values corresponding to the coldest winter surface temperature but will not yield values corresponding to the warmest summer surface temperature: this temperature will only be directly recoverable from 'supra-thermocline' individuals (i.e. those that lived above the depth of the summer thermocline).

Fig. 1 about here – double column

Sub-thermocline shells can still be a source of information on summer surface temperature: we can project a likely summer surface value by taking account of the difference between the maximum seafloor and surface temperature in modern stratified situations. This difference is variable (see Section 2) so sub-thermocline shells can only supply a rough indication of summer surface temperature, and before we add a 'stratification factor' to the warmest δ^{18}O-derived temperature from an individual, we must be sure that it lived in a sub-thermocline setting. Schöne and Fiebig (2009) claimed that sub-thermocline forms of the long-lived bivalve *Arctica islandica* are recognizable from a saw-tooth δ^{18}O profile (contrasting with a sinusoidal profile in supra-thermocline shells), but this pattern is also observed in an undoubtedly supra-thermocline individual (6 m depth) from south-west Scotland (Foster et al., 2009). Seafloor temperature variation can be sinusoidal in sub-thermocline settings (Fig. 1B) so under those circumstances the shells of bivalve species which grow throughout the year (e.g. many examples of the short-lived Queen Scallop, *Aequipecten opercularis*, in their first year; Hickson et al., 2000) would provide δ^{18}O profiles of the same form as supra-thermocline individuals.

Fig. 2 about here – single column

Using data from modern *Spisula solidissima*, Arthur et al. (1983) suggested that sub- and supra-thermocline bivalves might be distinguishable on the basis of patterns of covariation between $\delta^{13}C$ and $\delta^{18}O$: positive covariation (i.e. in-phase ontogenetic profiles) in the former and negative covariation (i.e. antiphase ontogenetic profiles) in the latter. This notion is superficially appealing because the $\delta^{13}C$ of dissolved inorganic carbon (DIC) increases over spring and summer at shallow depths due to the preferential uptake of ^{12}C by photosynthesizers, and then progressively decreases over the rest of the year, in part as a result of respiratory return of isotopically light carbon to the DIC pool and low photosynthetic uptake (Lorrain et al., 2004). Thus a supra-thermocline bivalve inheriting its shell carbon from DIC would show a pattern of seasonal variation in $\delta^{13}C$ opposite to that in $\delta^{18}O$. By contrast, a sub-thermocline bivalve might be expected to show parallel variation of $\delta^{13}C$ and $\delta^{18}O$ because DIC with a high $\delta^{13}C$ would only be available to it following the autumn breakdown of stratification and mixing down of surface waters. The problem with this model is that some of the carbon in a bivalve's shell derives from its food (particulate organic carbon), which, being isotopically light (the direct or indirect result of photosynthesis) and typically abundant at shallow depths during summer as a consequence of high primary production, might be expected to cause low shell $\delta^{13}C$ in supra-thermocline individuals during that season (Chauvaud et al., 2011). Low summer $\delta^{13}C$ values have indeed been documented from shallow-water *Pecten maximus* (Chauvaud et al., 2011), contrasting with the data of Arthur et al. (1983) from shallow-water *S. solidissima* but matching results from other shallow-water examples of *S. soldissima* (Krantz et al., 1987, figs 4, 5). Whether the summer $\delta^{13}C$ reduction seen in deeper water (sub-thermocline) *S. solidissima* (Arthur et al., 1983) and also *Placopecten magellanicus* (Krantz et al., 1987, figs 6–9) is due to high incorporation of food-derived carbon into the shell remains unclear (at depth, food availability might be no greater in summer than winter, although feeding rate would probably be higher; cf. Ren et al., 2000). However, whatever the cause, the foregoing examples indicate that summer reduction in shell $\delta^{13}C$ is not limited to sub-thermocline settings. The notion that sub- and supra-thermocline bivalves can be distinguished on the basis of patterns of $\delta^{13}C$ variation in relation to $\delta^{18}O$ is also refuted by data from *A. opercularis*. Modern examples from each setting (Fig. 2A, B) show patterns that are opposite to those recorded by Arthur et al. (1983) from *S. solidissima*, and some early Pliocene specimens of *A. opercularis* simply show a general ontogenetic decline in $\delta^{13}C$ (Fig. 2C).

Fig. 3 about here – double column

Included in Figure 2 are profiles of variation in the size (anatomical height) of microgrowth increments (Fig. 3). Smoothed (5-point average) data from the modern supra-thermocline shell (from the southern North Sea) exhibit a slight long-term fluctuation (broadly seasonal from the rough correspondence to the pattern of seasonal change in $\delta^{18}O$) superimposed on high frequency/low amplitude variation. In contrast the modern sub-thermocline shell (from the Gulf of Tunis, southern Mediterranean Sea) shows a much more pronounced long-term fluctuation, and the early Pliocene shell (from the Ramsholt Member, Coralline Crag Formation, Suffolk, eastern England) an essentially identical pattern. Investigation of 18 other *A. opercularis* from the eastern margin of the North Atlantic (including North Sea) revealed limited increment variation in all nine from definitely supra-thermocline settings, as evidenced by a macrotidal regime (hence strong tidal currents) or a depth of less than 25 m (Johnson et al., 2009, tables 1, 2). By contrast, two of four other investigated sub-thermocline shells from the Gulf of Tunis exhibit pronounced variation (> 0.30 mm between the minimum and maximum of smoothed profiles), as do four of seven other Ramsholt-Member shells (Johnson et al., 2009; Vignols et al., 2019). A sub-thermocline situation for Ramsholt-Member shells is supported by a variety of palaeontological and sedimentological evidence (Johnson et al., 2009), and the modest fluctuations seen in Ramsholt-Member $\delta^{18}O$ profiles (e.g. Fig. 2C) and quite low (cool temperate) peak temperatures calculated from these (contrasting with the warm temperate summer values indicated by the pelagic dinoflagellate assemblage; Head, 1997, 1998) are consistent with this setting (Johnson et al., 2009; Vignols et al., 2019). It cannot be denied, however, that the evidence for hydrographic control of increment-size variation in modern *A. opercularis* is only circumstantial, and that the $\delta^{18}O$ data from the Ramsholt Member could (if from supra-thermocline shells) reflect a low seasonality climate with cool summers. While such an interpretation would be radically at odds with almost all other evidence (Johnson et al., 2009; Vignols et al., 2019), it deserves consideration through further investigation of increment patterns in modern *A. opercularis* in relation to hydrographic setting, and of the closeness of shell $\delta^{18}O$ values to expected values. To this end we studied six further sub-thermocline shells live-collected for the purpose from another location in the Mediterranean Sea (northern Adriatic Sea, Croatia), temporally aligning shell $\delta^{18}O$ values as well as possible with a profile of predicted daily values derived for this location using the extensively validated Regional Ocean Modeling System (ROMS; Janeković et al., 2010, 2014; Vilibić et al., 2016) and a local water $\delta^{18}O$-salinity relationship (Peharda et al., 2019). We also supplemented the already quite large supra-thermocline database with information from two shells collected in the English Channel near Brighton, UK, an area from which data had not previously been obtained. In addition, we acquired isotope data from two probably supra-thermocline shells collected from the shore of the Mediterranean Sea at La Franqui, France. One of these had previously supplied increment data consistent with a supra-thermocline setting but the other (remeasured here) had yielded an anomalous (i.e. sub-thermocline) increment pattern. Since the precise location, depth and time of life of the English Channel and French Mediterranean individuals (museum specimens) was not known, we did not derive profiles of predicted shell $\delta^{18}O$ for comparison with measured values. Instead, we calculated summer and winter temperatures from the latter and compared these with mean seasonal temperatures at each location from direct monitoring.

Fig. 4 about here – single column

2. Material and settings

The general provenance of the Adriatic, English Channel and French Mediterranean shells (and those represented in Fig. 2) is illustrated in Figure 4; details of sample locations are supplied below and in Table 1.

The Adriatic shells are all left valves: S3A1, S3A3–5, S3A33 and S3A36 = University of Derby, Geological Collections (UD) 53417–53422, respectively. They are amongst the largest (47–51 mm shell height) from a sample of 54 individuals (27–53 mm height; uninvestigated specimens accessioned as UD 53423) live-collected with a dredge on 13th September 2016 by commercial fishermen at 38 m depth in fishing zone A3, 3.5 nautical miles from Pula, Croatia. Specimens were disarticulated and eviscerated shortly after collection. Valves were then matched and inscribed with a code-number (S3A1–54; same for right and left valve of a pair) before dry storage. We chose left valves for investigation because microgrowth increments are usually measurable over a greater range of shell height; the right valve, which lies against the substrate in life, can become significantly abraded, obscuring increment boundaries. ROMS-derived profiles of daily temperature for the surface and 38 m at the collection location show that the shells are unquestionably from a setting with summer thermal stratification, annual peak temperatures at 38 m being 3.2–9.9°C (mean 7.7°C) below those at the surface over the period 2008–2016 (Fig. 5A). This is a reflection of high heat flux and limited wave-mixing in summer, with tidal current velocities < 0.15 m s^{-1} (Chavanne et al., 2007); geostrophic currents are similarly weak (Djakovac et al., 2015). Surface temperatures are within the warm temperate range (winter > 10°C, summer > 20°C; Vignols et al., 2019).

Fig. 5 about here – single column

The English Channel shells are also left valves: EC1 and EC2 = Natural History Museum, London (NHMUK) 20190467/1 and 20190467/2, respectively. They are examples of similar size (53 and 52 mm height, respectively) to the Adriatic shells, selected from a sample originally formed of seven articulated individuals and five single valves (42–58 mm shell height; uninvestigated specimens accessioned as NHMUK 20190467) collected ‘off Brighton’ (south coast of the UK) on an unspecified date in 1923 and stored dry (without soft-parts). EC2 still had the right valve attached and EC1 could be matched with a right valve of the same size, so both the individuals concerned were probably alive at or not long before the time of collection. Since water depth is less than 20 m to 10 km offshore from Brighton and the surface velocity of tidal currents in the area exceeds 0.5 m s^{-1} at spring tides (VisitMyHarbour, 2012), it can be confidently assumed that the shells derive from a location where the annual peak temperatures at the seafloor and surface are (and were in the early 20th century) much the same—i.e. a setting without summer stratification. Mean seasonal extreme surface temperatures at Brighton are 8.0°C for winter and 17.4°C for summer (Global Sea Temperature, 2020)—i.e. within the cool temperate range (winter < 10°C, summer < 20°C; Vignols et al., 2019).

The French Mediterranean shells are a right valve 51 mm in height and a left valve 60 mm in height: FM1 and FM2 = Muséum National d’Histoire Naturelle, Paris (MNHN) IM-2008-1534 and IM-2008-1535, respectively. These are two of the three single valves from La Franqui (the other a right valve 53 mm in height: FM3 = MNHN-IM-2008-1533) for which increment data were supplied by Johnson et al. (2009, table 2). The three specimens were part of a sample of 35 single but unbroken valves (10–60 mm in height; uninvestigated specimens not given MNHN numbers) collected from the beach north of La Franqui on 25th and 31st October 1987 after a storm and stored dry (without soft-parts). It seems unlikely that a storm would have thrown onto the shore valves from more than a very few tens of metres depth—i.e. they were probably from supra-thermocline individuals. The low increment-size variation of FM1 and FM3 (respectively, 0.22 and 0.26 mm between the maximum and minimum of smoothed profiles) accords with this but FM2 provided a much higher value (0.36 mm), like that of sub-thermocline shells from the Gulf of Tunis (Johnson et al., 2009, table 2). Mean seasonal extreme surface temperatures at La Franqui are about 12°C for winter and 23°C for summer (i.e. within the warm temperate range), but while the cool extreme is the same down to 30 m, the warm extreme declines gradually to about 20°C at 30 m and then somewhat more rapidly to about 17°C at 50 m (NOAA, 1994).

3. Methods

3.1. Laboratory procedures

Shells were coated with a sublimate of ammonium chloride and digitally photographed, then images were inserted into the bespoke software Panopea© (2004, Peinl and Schöne) for the purposes of increment measurement, to the extent that this could be accomplished (increments were usually invisible in the umbonal area, and sometimes elsewhere, due to abrasion). The coating was washed off with tap-water and most shells then underwent the further cleaning procedure adopted by Valentine et al. (2011) for removal of any surficial organic material prior to isotopic sampling. One Adriatic shell (S3A4) and the French Mediterranean shells were sampled before it was decided to implement this procedure. Samples for isotope analysis were obtained by drilling a dorsal to ventral series of shallow (< 1 mm deep) commarginal grooves into the outer surface of the outer (calcite) shell layer, with the sample sites more closely spaced towards the ventral margin in an attempt to maintain temporal resolution in the context of declining growth rate with age. Details of the measurement and sampling methods used, including the adjustment procedure employed where it was impossible to measure or sample along the dorso-ventral (height) axis, are given in Johnson et al. (2019) with respect to another scallop species. All the increment measurements were made by the same person (AMV) to achieve as uniform an approach as possible, given that increment identification can locally be a subjective matter.

Increments are commonly difficult to define between the plicae ('ribs') on left valves (Fig. 3B, C); measurements were made on the plicae in such circumstances. Growth breaks were identified as minor, moderate or major (the last two classes subsequently combined) dependent on the size of the characteristic 'step' in the shell profile, which marks a near or total cessation of extensional growth but not of shell thickening. Minor growth breaks are subtle features (Fig. 3A, B), sometimes only observable by tilting the shell to various angles. The height of growth breaks was therefore determined on the shells themselves (with a ruler) rather than on digital images.

In cool temperate representatives of *A. opercularis*, such as those from around the UK, prominent growth breaks typically occur in winter and appear to represent intervals of a month or two (e.g. Broom and Mason, 1978). However, in other scallop species they may occur at any time of year (e.g. Johnson et al., 2019), presumably reflecting disturbance in some cases, and intervals approaching six months may be represented (Krantz et al., 1984). No assumptions can therefore be made about the timing or duration of growth breaks in warm temperate *A. opercularis*, such as Adriatic individuals, to guide general alignment of shell δ^{18}O values with predicted values. However, from isotopic evidence of growth slowdown or cessation for several summer months in Adriatic *Pecten jacobaeus*, and of the same for several winter months in Adriatic *Glycymeris pilosa* (Peharda et al., 2019), it is reasonable to expect some attenuation of growth in Adriatic *A. opercularis* during one or both seasons, and to use evidence of this in the form of growth breaks to refine alignments based on identification of annual δ^{18}O cycles (see Section 3.2).

Table 1 about here - ? single column

Stable oxygen and carbon isotope analysis (given as δ^{18}O and δ^{13}C) were carried out either at the stable isotope facility, British Geological Survey, Keyworth, UK, or at the Institute of Geosciences, University of Mainz, Germany. At Keyworth samples were analysed using an Isoprime dual inlet mass spectrometer coupled to a Multiprep system; powder samples were dissolved with concentrated phosphoric acid in borosilicate Wheaton vials at 90°C. At Mainz samples were analysed using a Thermo Finnigan MAT 253 continuous flow-isotope ratio mass spectrometer coupled to a Gasbench II; powder samples were dissolved with water-free phosphoric acid in helium-flushed borosilicate exetainers at 72°C. Both laboratories calculated $\delta^{13}C$ and $\delta^{18}O$ against VPDB and calibrated data against NBS-19 (preferred values: +1.95‰ for $\delta^{13}C$, –2.20‰ for $\delta^{18}O$) and their own Carrara Marble standard (Keyworth: +2.00‰ for $\delta^{13}C$, –1.73‰ for $\delta^{18}O$; Mainz: +2.01‰ for $\delta^{13}C$, –1.91‰ for $\delta^{18}O$). Values were consistently within ± 0.05‰ of the values for $\delta^{18}O$ and $\delta^{13}C$ in NBS-19. Reproducibility was checked by duplicate analysis of some samples, and in the case of seemingly aberrant initial results, repeat sampling and analysis was undertaken. The profiles in Figures 7–9 link singleton values and the means of multiple values. In two cases where results from repeat analysis differed greatly (S3A5, height 46.5 mm; EC2, height 16.5 mm) the more credible data have been used for the profiles and suspect data omitted. Otherwise, all the isotope results obtained are plotted in Figures 7–9.

The full set of raw isotope and increment data, together with modelled values of temperature, salinity and expected shell $\delta^{18}O$ (Section 3.2), is available online (see Appendix A). A summary of the isotope data is provided in Table 1.

3.2. Calculations of temperature and expected shell $\delta^{18}O$

In earlier isotope studies of modern and fossil *A. opercularis* (Hickson et al., 1999, 2000; Johnson et al., 2000, 2009; Valentine et al., 2011; Vignols et al., 2019) the calcite equation of O'Neil et al. (1969) was used to calculate temperatures from shell $\delta^{18}O$ or to calculate expected shell $\delta^{18}O$ values from known temperatures, in each case with an adjustment (most recently –0.27‰, following Gonfiantini et al., 1995) to convert water $\delta^{18}O$ values from the VSMOW scale to the VPDB scale used for calcite. In isotope work on *P. jacobaeus* from the northern Adriatic, Peharda et al. (2019) used the more recent calcite equation of Kim and O'Neil (1997), also widely employed in studies of other calcitic taxa. The equation of Kim and O'Neil (1997) yields temperatures about 2°C lower than that of O'Neil et al. (1969) so the choice of equation is a significant matter. Other calcite equations exist, one of which—the LL (low light) equation of Bemis et al. (1998)—was used by Austin et al. (2006, fig. 6) to derive expected values for calcite $\delta^{18}O$ over a year from monthly data on bottom temperature and water $\delta^{18}O$ for the same latitude (53°N) in the southern North Sea as the *A. opercularis* shell represented in Figure 2A. We used the data of Austin et al. (2006) to also derive expected values of shell $\delta^{18}O$ using the equations of O'Neil et al. (1969) and Kim and O'Neil (1997), and then replicated the three sets of values to produce multi-year profiles (Fig. 6) for comparison with the data from the southern North Sea *A. opercularis* specimen. The most suitable equation was judged by temporally aligning the shell data as well as possible with each profile (i.e. minimising the overall difference between measured and expected shell values within a three-year period, as specified by the number of $\delta^{18}O$ cycles), and then reviewing the closeness of each fit. During early ontogeny, growth of *A. opercularis* may be rapid and continuous through summer and winter in the southern North Sea (Johnson et al., 2009). From the long 'wavelength' of the first $\delta^{18}O$ cycle (measured versus shell height) and the absence of visible growth breaks associated with the lowest and highest values (Fig. 2A), it is evident that the present specimen did grow rapidly and without interruption during early ontogeny. The most extreme $\delta^{18}O$ values measured over the first cycle can therefore be taken to represent the most extreme temperatures experienced, and the equation yielding predicted $\delta^{18}O$ values closest to those measured can be regarded as the best descriptor of the relationship between temperature and shell $\delta^{18}O$. The LL equation of Bemis et al (1998) provides the closest values (Fig. 6), the equation of Kim and O'Neil (1997) yielding values that are somewhat lower than the lowest measured value from the first summer and the equation of O'Neil et al. (1969) yielding values that are somewhat higher than the highest measured value from the first winter. For this reason we used the

LL equation of Bemis et al. (1998) to derive expected values of shell δ^{18}O and to calculate temperatures from the other *A. opercularis* shells investigated herein.

Fig. 6 about here – single column

ROMS-derived daily salinity values (Fig. 5B) and the salinity-water δ^{18}O relationship established by Peharda et al. (2019) for adjacent sites were used to calculate daily water δ^{18}O values (Fig. 5B) for the location and depth of the Adriatic shells. These values and the modelled daily seafloor temperatures (Fig. 5A) were then used in the equation of Bemis et al. (1998) to calculate values of expected shell δ^{18}O (Fig. 5C) for comparison with data from *A. opercularis*. This mirrors the approach of Peharda et al. (2019) for other locations and depths in the northern Adriatic, except that they employed the equation of Kim and O'Neil (1997) to derive expected values of shell δ^{18}O for comparison with data from *P. jacobaeus*.

In view of the inadequate contextual information we did not attempt to derive expected daily values of shell δ^{18}O for comparison with data from the French Mediterranean and English Channel shells. Instead we compared local summer and winter temperatures (Section 2) with the warmest and coolest temperatures calculated from shell δ^{18}O, using the LL equation of Bemis et al. (1998) and a representative single value of water δ^{18}O from measurements made nearby: +1.3‰ for La Franqui, based on measurements at 4–25 m depth in the north-western Mediterranean (site 1B of Pierre, 1990); +0.2‰ for the English Channel near Brighton, based on measurements in the North Sea (Mook and Vogel, 1968).

4. Results

4.1. Adriatic shells (Fig. 7)

Fig. 7 about here – double column

All $\delta^{18}O$ profiles show between one and two major cycles, the amplitude and (in most cases) wavelength decreasing in the second cycle, where present. By contrast, $\delta^{13}C$ profiles show little variation, values tending to decrease slightly over the course of ontogeny (e.g. Fig. 7F) and occasionally increasing over short intervals in association with decreasing $\delta^{18}O$ (Fig. 7C, E), but otherwise showing no relation to this parameter. In that the isotope data from S3A4 (Fig. 7C) are closely comparable with those from other shells, it appears that omission of the procedure to remove surficial organic material from the shell had little or no effect (cf. Schöne et al., 2017). In all cases apart from S3A1 (Fig. 7A) the smoothed increment profiles exhibit one more-or-less clear major cycle, with a difference of more than 0.30 mm between the maximum and minimum values. The absence of such features from the S3A1 profile could be because it is the shortest of the six. Amongst the five individuals showing marked variation in increment size, the maximum size is observed in mid-ontogeny, and occurs in an interval of decreasing or low $\delta^{18}O$ in four specimens (S3A3, S3A4, S3A5, S3A33) and of increasing $\delta^{18}O$ in one (S3A36). The minimum size is shown in early or late ontogeny and occurs in an interval of decreasing or low $\delta^{18}O$ in four specimens (S3A3, S3A4, S3A5, S3A36) and of high $\delta^{18}O$ in one (S3A33). Growth breaks are concentrated in late ontogeny and are commonly associated with the highest and lowest $\delta^{18}O$ values in this interval. Figure 3B illustrates growth breaks and the pattern and scale of increment variation in S3A4.

4.2. English Channel shells (Fig. 8)

Fig. 8 about here – double column

Both the $\delta^{18}O$ and $\delta^{13}C$ profiles of the English Channel shells are very similar in their general features to those from the (similarly sized) Adriatic shells. However, the $\delta^{18}O$ profiles of the former differ by including lower values and the $\delta^{13}C$ profiles by their consistently higher values at all stages of ontogeny (see also Fig. 11), with instances of positive (rather than negative) correlation with $\delta^{18}O$ (early ontogeny of EC1—Fig. 8A; late ontogeny of EC2—Fig. 8B). Smoothed increment profiles show a much smaller range of variation (well below 0.30 mm) than is seen in most Adriatic shells. Minimum values are much the same as in Adriatic shells but maximum values are less than in most Adriatic shells. Growth breaks are concentrated in late ontogeny; examples are associated with the $\delta^{18}O$ maxima (but not with the minima) in this interval in the two shells. Figure 3C illustrates the subdued increment variation in EC1.

4.3. French Mediterranean shells (Fig. 9)

Fig. 9 about here – double column

The $\delta^{18}O$ profiles are again very similar in their general features to profiles from Adriatic shells, spanning also a comparable range of values. However, in FM1, in which 1.5 cycles are identifiable, the amplitude (and wavelength) is greater in the second (incomplete) cycle. The $\delta^{13}C$ profiles are also quite similar in their general features to those from Adriatic shells, but with higher values at most stages of ontogeny (although not generally as high as from English Channel shells; see also Fig. 11) and instances of positive correlation with $\delta^{18}O$ (mid-ontogeny of FM1—Fig. 9A; late ontogeny of FM2—Fig. 9B), although there are also some intervals of negative correlation in FM1. In view of the evidence from Adriatic shell S3A4 it may be assumed that omission of the procedure to remove surficial organic material had no effect on the isotope data from the French Mediterranean shells. The smoothed increment profiles show variation of less than 0.30 mm (remeasurement of FM2 revealed that the anomalously high variation recorded previously was down to a single, very inaccurate value in the raw data). As in the English Channel shells, minimum increment sizes are much like those from Adriatic shells and maximum sizes are less than from most Adriatic shells. Growth breaks are more frequent in early ontogeny than in the Adriatic and English Channel shells; examples are associated with the δ^{18}O maxima (but not with the minima) in the two shells.

5. Interpretation

5.1. $\delta^{18}O$ data

The number of δ^{18}O cycles (one to two) in the profiles illustrated in Figures 7–9 is the same as in most profiles up to the same shell height from other modern (and fossil) *A. opercularis* (Hickson et al., 1999, 2000; Heilmayer et al., 2004; Johnson et al., 2009; Valentine et al., 2011; Vignols et al., 2019). Taking the cycles essentially to reflect seasonal variation in water temperature (cf. Section 3.2), and their generally declining wavelength to reflect declining growth rate, the implied ages and rates of growth-rate decline are consistent with growth statistics for *A. opercularis* obtained by other means (e.g. Taylor and Venn, 1978; Richardson et al., 1982; Heilmayer et al., 2004). It is thus clear that none of the shells were more than three years old. In the case of the Adriatic specimens, we can use this information to guide temporal alignment of measured δ^{18}O values with predicted values, following the same approach as for the southern North Sea specimen discussed in Section 3.2. We consider details of the alignment of Adriatic data immediately below, proceeding to evaluate the extent to which shell δ^{18}O profiles from this area reflect ambient temperature. We then discuss δ^{18}O-derived temperatures from English Channel and French Mediterranean shells in more general terms—i.e. the fidelity of summer and winter values to mean seasonal temperatures.

Fig. 10 about here - double column

5.1.1. *Adriatic shells*

Figure 10 shows predicted shell $\delta^{18}O$ for a period spanning the lives of the Adriatic shells (i.e. from late 2016 as far back as 2013), accompanied by aligned measured values from each shell. It has been assumed that growth breaks adjacent to the locations of maxima and minima in measured values relate to extreme winter and summer temperatures—i.e. the maximum/minimum $\delta^{18}O$ values concerned have been positioned so that the adjacent growth break includes the time of inflection in the curve of predicted values (cf. Section 3.1).

General positioning of values (i.e. assignment to years) was largely unproblematic except in the case of S3A1, where two significantly different 'solutions' emerged: one with most values assigned to 2015, and another with most values assigned to 2014. The latter achieves an alignment that is slightly more precise overall but leaves a gap of 188 days in summer 2015. Unlike the similarly long gaps in summer 2015 in S3A4 (187 days) and S3A36 (174 days) there is no growth break to explain this gap so the former alignment (which leaves a gap of 49 days, unaccompanied by a growth break, in summer 2015) has been used in Figure 10A. Adoption of the alternative alignment for S3A1 would not have affected the argument below. The long gaps (> 100 days; identified by grey bars) in other records are unavoidable; alternative arrangements to reduce them result in larger, inexplicable gaps elsewhere. Some of them are associated with growth breaks but not the winter 2014 gaps in S3A5 and S3A36, and the gaps from spring (or even late winter) 2016 to the date of collection shown by all shells except S3A1 (in which there is nevertheless a shorter gap). The winter 2014 gaps relate to the abraded umbonal areas of the shells concerned, where it was impossible to obtain growth-increment data, and evidence of growth breaks may therefore have been lost. The 2016 gaps probably reflect the culmination of ontogenetic decline in growth rate, with the last sample from each shell (taken at or very close to the shell edge) including a significant amount of material deposited early in the year. The long gaps can therefore be explained by time-averaging of sample material and provide no grounds for suspecting serious misalignment—i.e. with the wrong year.

Multiple, similar values within a short time interval (e.g. S3A5 and S3A33, mid-summer 2015) might be regarded as evidence of serious misalignment because they demand very rapid growth. However, the rates required are not unreasonable. In the examples cited the height intervals between the first and last values are 6.0 and 5.5 mm, respectively (Fig. 7D, E), amounts of size increase that have been shown through experiment to be achievable by *A. opercularis* in a month or somewhat less under warm conditions (Broom and Mason, 1978, table 2). This period is comparable to the time interval of 20 days spanned in each case by the mid-summer 2015 values from S3A5 and S3A33, as positioned in Figure 10. In S3A33 a period of 10 days in spring 2015 is represented by a series of five $\delta^{18}O$ values, which align perfectly with the plot of predicted values and cover a height interval of 9.5 mm (Fig. 7E), confirming the plausibility of a size increase of 5.5 mm in 20 days during the succeeding summer.

While it appears that the $\delta^{18}O$ values from all shells have been associated with the correct year, not every value can be precisely aligned with the curve of predicted values. Small (unavoidable) discrepancies can be considered to reflect the known analytical and modelling errors. Moderate departures (< 0.5‰) from predicted values, for example in summer 2014 (S3A3, S3A4, S3A36; all negative) and winter 2016 (S3A33, S3A36; both positive), seem likely to reflect inaccuracy in the shell record of ambient conditions (i.e. non-equilibrium isotope incorporation), and are discussed further below. Large departures (> 0.5‰; open symbols) from predicted values in summer 2014 (S3A5; positive), winter 2015 (S3A3, S3A4, S3A5, S3A33; all negative) and spring 2016 (S3A1; positive) are unlikely to have this cause, given the quite close correspondence of measured to predicted values in *A. opercularis* from the southern North Sea (Fig. 6). Contamination by material from other sample positions can be ruled out because all the significantly discrepant values were checked by resampling, either at the place of the initial sample or very close by. That four shells show a confirmed, large discrepancy of the same sign in winter 2015 (and that S3A36 shows a smaller discrepancy of the same sign) suggests that the departure from expectation is a function of inaccurate modelling—i.e. that this failed to take into account some environmental event in winter 2015. While in theory the observed negative excursion could reflect a brief warming episode, a winter influx of (isotopically light) freshwater is a more plausible event. The River Po is the likeliest source, given its large supply of freshwater to the northern Adriatic in general (Vilibić et al., 2016, table 1). The confirmed, large, positive departures are difficult to account for, particularly the summer 2014 excursion in S3A5, which could exceed 1.0‰, dependent on precise timing. Being isolated instances, these departures perhaps reflect incorporation of carbonate from some small, unnoticed encrusting organism.

Of those (moderate) departures from predicted values which seem likely to reflect non-equilibrium isotope incorporation, the largest in summer 2014 (0.31‰) is from S3A3 and the largest in winter 2016 (0.28‰) is from S3A33. The former departure translates into a temperature overestimate of 1.5°C and the latter into an underestimate of 1.3°C. While these figures give a good indication of the extent by which seasonal temperatures calculated from *A. opercularis* shell $\delta^{18}O$ might be exaggerations of the temperature extremes experienced, they do not represent amounts which can be routinely combined with isotopic summer and winter temperatures to determine actual seasonal extremes. This is because the extreme measured values of shell $\delta^{18}O$ are often not as low in summer and not as high in winter as the extreme predicted values. Thus, in addition to the large gaps referred to above, there are smaller gaps in the shell records (winter 2015 in S3A36; summer 2015 in S3A1, S3A3, S3A5 and S3A33) which omit values corresponding to the relevant predicted extremes. In the case of winter 2015 in S3A36 the discrepancy between measured and predicted extreme values is fairly small (0.29‰; equivalent to a temperature overestimate of 1.4°C). However, in the summer 2015 cases the discrepancies are substantial (largest 0.98‰ in S3A3; equivalent to a temperature underestimate of 4.7°C). In most cases there are associated growth breaks (possible environmental cause discussed in Section 5.4), so it is no surprise that the shell records are incomplete. However, the magnitude of the discrepancies with the predicted δ^{18}O extreme for summer 2015, within datasets for that season which are otherwise fairly complete, is a salutary demonstration of the potential for misinterpretation of summer temperatures from *A. opercularis* shell δ^{18}O. Of the four individuals alive in summer 2014 as well as summer 2015 (S3A3, S3A4, S3A5, S3A36) only S3A5 shows a lower minimum δ^{18}O value for 2015 than for 2014, despite the predicted minimum for summer 2015 (–0.21‰) being much lower than for 2014 (+0.37‰) as a consequence of the unusually warm conditions (Fig. 5). This suggests that the incompleteness of the summer 2015 records from these shells might be a function of their age and consequent slower growth, leading to greater time-averaging within samples (cf. Fig. 6). However, the summer 2015 records from S3A1 and S3A33, individuals which were not alive in summer 2014, are similarly incomplete. In S3A33 a growth break is associated with the lowest measured value of δ^{18}O for summer 2015, providing a hint that the individual experienced warmer temperatures than are recorded in its δ^{18}O profile. However, the discrepancy between the measured and predicted extremes (0.83‰; equivalent to a temperature underestimate of 3.9°C) is not much less than in the ontogenetically older shell S3A3 (see above), and the discrepancy in S3A1 (0.97‰; equivalent to a temperature underestimate of 4.6°C), a shell which shows no growth break in summer 2015, is very nearly the same as in S3A3. It therefore appears that neither restricting sampling to early ontogeny nor examining specimens for the existence of growth breaks are strategies which could lead to accurate estimation of summer temperature every year in the Adriatic from the shell δ^{18}O of *A. opercularis*. There is potential for serious underestimation of the peak temperature in warm years (evidenced by the data for 2015), although in cooler years (evidenced by the data for 2014) δ^{18}O records are fairly accurate, tending to provide just a slight overestimate (< 1.5°C) of the warmest temperature. The situation is otherwise for winter temperature, conditions in both 2015 and 2016 being fairly accurately represented by at least some shells, these providing a slight overestimate (1.4°C) of the coolest temperature for the former year and a slight underestimate

(< 1.3°C) for the latter. Turning these findings into guidelines for interpreting $\delta^{18}O$ records from sub-thermocline *A. opercularis* in general, one should only regard minimum $\delta^{18}O$ values as an indication of seafloor temperature in relatively cool summers. Maximum $\delta^{18}O$ values can, however, be regarded as an indication of average winter seafloor temperature, provided that data are available from at least a few shells, and anomalously low values associated with growth breaks are excluded from consideration.

5.1.2. English Channel and French Mediterranean shells

Summer and winter inflections can be recognised in the English Channel and French Mediterranean $\delta^{18}O$ profiles (Figs 8, 9) at the following shell heights. EC1— winter 1: 5.5 mm, summer 1: 38.0 mm, winter 2: 49.5 mm; EC2—summer 1: 16.5 mm, winter 1: 39.0 mm; summer 2: 49.0 mm; FM1—summer 1: 9.0 mm, winter 1: 25.0 mm, summer 2: 48.0 mm; FM2—winter 1: 29.0 mm, summer 1: 41.5 mm. While there are no growth breaks associated with $\delta^{18}O$ minima to suggest truncation of summer records, the association of growth breaks with almost all $\delta^{18}O$ maxima (winter 1 in EC1 is the sole exception) suggests possible truncation of winter records. However, given the lengthy upward and downward trends on either side of each $\delta^{18}O$ maximum, which can be taken to span the preceding autumn and succeeding spring periods, any truncation is likely to have been minor.

The summer 1 isotopic temperatures (from $\delta^{18}O$ minima) in the English Channel shells (EC1: 18.2°C; EC2: 18.8°C) are similar to the local average warm extreme (17.4°C), but consistently a little warmer. The rather cool summer 2 temperature in EC2 (14.8°C) could reflect sampling at inadequate resolution (i.e. closer sampling later in ontogeny failed to compensate for declining growth rate; cf. Fig. 6). All the winter temperatures (from $\delta^{18}O$ maxima) in EC1 (winter 1: 6.1°C; winter 2: 7.2°C) and EC2 (winter 1: 7.2°C) are similar to the local average cool extreme (8.0°C), but consistently a little cooler. As growth breaks are associated with the two warmer (7.2°C) recorded extremes it is possible that the records are a little truncated—i.e. that the actual minimum temperatures in the winters concerned were similar to the cooler (6.1°C) recorded extreme. Like the winter temperatures from the English Channel shells, those from the French Mediterranean shells (FM1, winter 1: 12.9°C; FM2, winter 1: 10.3°C;) are similar to the local average cool extreme (*c.* 12°C), but while one is a little cooler, the other is a little warmer. As growth breaks are associated with both recorded extremes, the actual minimum temperatures in the winters concerned may have been a little lower. All the summer temperatures in FM1 (summer 1: 21.2°C; summer 2: 21.7°C) and FM2 (summer 1: 19.9°C) are cooler than the local average warm extreme at the surface (*c.* 23°C). This seems likely to reflect life-positions near the base of the mixed layer, at depths down to 30 m, where the average peak temperature is 20°C. This would not be inconsistent with transport to the shore during a storm.

To summarize and conclude, winter temperatures calculated from the $\delta^{18}O$ of the English Channel and French Mediterranean shells are quite close to expectation; values cooler than the local average (the majority) might represent underestimates of actual winter temperatures due to disequilibrium, but could be essentially accurate, reflecting relatively cool winters. Summer temperatures are in some cases quite close to expectation but in others several degrees cooler; the latter instances might also represent disequilibrium but seem more likely to be effects of growth-rate decline with age or life in relatively deep (but not sub-thermocline) settings. The English Channel and French Mediterranean shells therefore provide a fairly accurate indication of average winter temperatures, like the Adriatic shells, but unlike the latter they also provide (or would be capable of providing if suitably sampled) a fairly accurate indication of average summer benthic temperatures.

5.2. $\delta^{13}C$ data

Of the three sets of shells, only the French Mediterranean specimens show any sign of the pattern of $\delta^{13}C$-$\delta^{18}O$ covariation predicted for their hydrographic setting from the data and arguments of Arthur et al. (1983). The intervals of negative correlation in FM1 are consistent with the evidence of relatively warm summer temperature from this shell indicating a supra-thermocline setting. However, the interval of positive correlation is inconsistent. The interval of positive correlation in FM2 is consistent with the evidence of relatively cool summer temperature from this shell suggesting a life-position a little below the mixed layer, although only an intra- rather than truly sub-thermocline setting is permissible from the temperature evidence. Far from showing the predicted positive correlation between δ^{13}C and δ^{18}O (i.e. in-phase variation), some of the Adriatic (sub-thermocline) shells show instances of negative correlation. Likewise, instead of showing a negative correlation (i.e. antiphase variation), both of the English Channel (supra-thermocline) shells show instances of positive correlation.

Fig. 11 about here – single column

The widely exhibited ontogenetic decline in δ^{13}C values is as seen in many bivalves and may well reflect greater use of carbon released by the organism's respiration (isotopically light) for shell formation with increasing age (Lorrain et al., 2004; McConnaughey and Gillikin, 2008; Chauvaud et al., 2011). The geographic differences in δ^{13}C values (Adriatic < French Mediterranean < English Channel; Fig. 11) resemble those revealed by Chauvaud et al. (2011, fig. 3) in *Pecten maximus* from coastal locations in the eastern North Atlantic (Spain < France < Norway). Those in *P. maximus* probably relate in part to variation in the amount of respiratory carbon used for shell formation as determined by temperature, but differences in the amount of food consumed, and its carbon isotopic composition, also influence δ^{13}C in this species (Marchais et al., 2015). While the Adriatic *A. opercularis* certainly lived and grew under warmer winter conditions than the English Channel individuals, the French Mediterranean individuals did so under much the same winter temperatures, and actually grew under warmer summer conditions than the Adriatic shells. Differences in shell δ^{13}C are not therefore matched by temperature differences and probably relate mainly to nutrition.

5.3. Microgrowth-increment data

The variation in (smoothed) increment size of more than 0.30 mm shown by five of the six Adriatic shells is as predicted for their sub-thermocline setting, and the variation of less than 0.30 mm shown by both English Channel shells is as predicted for their supra-thermocline setting. Viewed as essentially supra-thermocline (i.e. including uppermost intra-thermocline settings but no deeper), the variation in increment size of less than 0.30 mm shown by the French Mediterranean shells is also as predicted.

The large increments in mid-ontogeny that account for the greater size variation in sub-thermocline Adriatic shells are associated with declining, low or increasing δ^{18}O values—i.e. with times in the spring–autumn period. They seem likely to reflect high food availability then in the form of phytoplankton. The small increments in early and late ontogeny, often also associated with declining or low δ^{18}O values, may reflect overriding age effects. The lack of large increments in supra-thermocline English Channel and French Mediterranean shells cannot be attributed to a lack of phytoplankton in the spring–autumn period. Possibly these individuals subsisted largely on resuspended detritus (cf. Johnson et al., 2009). The availability of this throughout the year as a result of wave and current action would account for the minimal variation in increment size, and its low nutritive value would explain the small absolute size of increments.

5.4. Synthesis

It is clear from the foregoing evidence that the pattern of variation in δ^{13}C relative to δ^{18}O cannot be used to distinguish sub- from supra-thermocline examples of *A. opercularis*, but that the pattern of variation in (smoothed) microgrowth-increment height can, at least if a number of specimens are examined to allow for occasional departures from the norm of large-amplitude (> 0.30 mm) variation over the course of ontogeny in sub-thermocline forms. One qualification is necessary: while the increment results from Adriatic sub-thermocline individuals agree with those obtained previously from Gulf of Tunis specimens, it should be recognised that both sets derive from warm temperate environments. Almost all the available increment information from modern cool temperate shells (Johnson et al., 2009, tables 1, 2) is definitely or probably from supra-thermocline settings, and the two specimens that most likely derive from sub-thermocline settings (mesotidal but *c.*110 m depth in the Firth of Clyde, Scotland and Western Approaches, Ireland/England) actually exhibit little variation in increment height. It is possible therefore that the large-amplitude variation seen in a high proportion of Mediterranean (warm temperate) sub-thermocline *A. opercularis* is not characteristic of cool temperate sub-thermocline forms.

Winter temperatures are quite accurately registered (to within 2°C) by the δ^{18}O of sub-thermocline shells from the Adriatic, as seemingly by English Channel and French Mediterranean supra- and intra-thermocline shells. However, while English Channel and French Mediterranean shells probably provide (or could provide) a fairly accurate record of summer benthic temperatures, Adriatic shells present a biased picture: they provide a fairly accurate estimate (to within 2°C) of the peak temperature in relatively cool years but a serious underestimate in warm years. Thus the warmest modelled seafloor temperature for the warm summer of 2015 is 23.3°C (Fig. 5A; Table 2) but the warmest isotopic temperature for 2015 (from S3A5) is 20.0°C, well short of this figure. However, the warmest isotopic temperature from the Gulf of Tunis sub-thermocline shell represented in Figure 2B is very close (22.7°C; calculated using a water δ^{18}O value of +1.35‰, measured at 50 m depth at an adjacent location—site 8 of Pierre, 1990). Hence *A. opercularis* is capable of recording temperatures similar to those reached in warm summers in the Adriatic but for some reason failed to do so there in 2015. Most shells suffered an interruption of growth in summer 2015, suggesting some unfavourable environmental condition. This might have been low quantity or quality of food (cf. Johnson et al., 2009), but hypoxia seems the likeliest cause, given its known effect on growth in bivalves (Gobler et al., 2014; Gobler and Baumann, 2016) and fairly common summer occurrence in the northern Adriatic area (Djakovac et al., 2015). Such evidence of summer benthic oxygen levels as exists in relation to the sample site near Pula (for the summers of 1972, 1977, 1983, 1989, 2003, 2006; Djakovac et al., 2015, fig. 2) indicates or strongly suggests full saturation. However, Kralj et al. (2019) recorded hypoxic events in the Bay of Trieste (*c*. 85 km north of Pula) during the exceptionally warm summers of 2015 and 2016, reversing a 30-year trend to increasing benthic oxygenation, so it is possible that hypoxia extended to the Pula area then. It would certainly be worth measuring benthic oxygen levels in the Pula area during future exceptionally warm summers, and documenting the growth of *A. opercularis* over these intervals. It would also be worth investigating the diet of sub- and supra-thermocline *A. opercularis* to see if, as speculated, this differs in sub- and supra-thermocline forms and provides an explanation for the high variation in increment size over the course of ontogeny in the former and low variation in the latter.

Table 2 about here - ? single column

6. Application to the early Pliocene Ramsholt Member

Our results from modern shells confirm that the occurrence of high-amplitude increment variation amongst examples of *A. opercularis* from the early Pliocene Ramsholt Member of eastern England is an indication of a sub-thermocline setting, as also suggested by other evidence. Therefore, while isotopic minimum temperatures can be read as surface minima, isotopic maximum temperatures cannot be read as surface maxima. The amount by which the peak in benthic temperature obtained from δ^{18}O data underestimates the surface maximum temperature is a matter of some uncertainty. Vignols et al. (2019) suggested a stratification factor of 5°C for the Ramsholt Member on the basis of modern temperature data from the Gulf of Tunis. However, as noted earlier, the difference between annual seafloor and surface temperature maxima in the northern Adriatic varies considerably, from 3.2−9.9°C over the nine years from 2008−2016. Adding the mean difference (7.7°C) to the seafloor maximum in each year yields, of course, an accurate figure for the mean surface maximum over this period (27.6°C; Table 2). However, while 7.7°C serves as an accurate average stratification factor for the interval, for the two individual years with the highest seafloor temperature maxima (2015: 23.3°C; 2016: 23.5°C), it provides significant overestimates of the respective surface maxima (by 1.5°C and 4.5°C), because the difference between the surface and seafloor maxima is least in these years (surface maximum 6.2°C higher than seafloor in 2015 and 3.2°C higher than seafloor in 2016; Table 2). We know, however, that while *A. opercularis* shell $\delta^{18}O$ provides a fairly accurate estimate of maximum seafloor temperature in years of relatively cool seafloor conditions (at least in 2014), it provides a 3−4°C underestimate in unusually warm years (at least in 2015). Adjusting the maximum (modelled) seafloor temperatures for 2015 and 2016 downward by an amount (3.5°C) reflecting this, then recalculating the mean surface maximum for 2008−2016 assuming the same stratification factor (7.7°C), yields a value of 26.8°C, only 0.8°C below the actual figure (Table 2). Thus, armed with a knowledge of water $\delta^{18}O$ and of the average stratification factor, we should be able to reconstruct the mean surface maximum temperature for any given period quite accurately from *A. opercularis* shell $\delta^{18}O$, irrespective of whether the period includes unusually warm years.

Water $\delta^{18}O$ and the average stratification factor cannot be precisely specified in 'fossil' situations, but they may be constrained. Before providing approximations for the Ramsholt Member, it is worth noting the effect of using incorrect, but plausible stratification factors in calculation of Adriatic surface temperature. If, instead of 7.7°C, we add 9.9°C (the largest difference between annual seafloor and surface maxima in the 2008−2016 period) to all the seafloor temperature values used previously, we obtain a figure of 29.0°C for mean surface maximum temperature, just 1.4°C above the actual figure; by contrast, if we add 3.2°C (the smallest difference between annual seafloor and surface maxima in the 2008−2016 period), we obtain a figure of 22.3°C, 5.3°C below the actual figure (Table 2). Thus what can be regarded as maximum and minimum stratification factors yield, respectively, a slight overestimate and a significant underestimate of the actual mean surface maximum temperature—i.e. in 'fossil' situations, we should favour estimates of surface maximum temperature based on stratification factors in the upper part of the 'plausible range'. What therefore might be this range for the Ramsholt Member? The lower limit must surely be above 2.6°C, the figure for the difference between the surface (13.7°C) and seafloor (11.1°C) maxima at the stratified North Sea site represented in Figure 1B. The surface maximum at this site (57°N), over 500 km north of the Ramsholt Member's location (*c.* 52°N), is far below the summer surface temperature of at least 20°C implied by the warm temperate pelagic dinoflagellate assemblage of the Ramsholt Member (Head, 1997, 1998). There has been a long history of also interpreting the Ramsholt Member's benthic biota as of warm-water aspect, and specifically 'Mediterranean' in the case of the Mollusca (Long and Zalasiewicz, 2011; Vignols et al., 2019). While shell $\delta^{18}O$ evidence argues strongly against warm temperate seafloor conditions, it is entirely consistent (assuming stratification) with summer surface temperatures in the warm temperate range (Johnson et al., 2009; Vignols et al. 2019). Stratification factors under such a climatic regime are therefore the best indication of the plausible range for the Ramsholt Member, and the 3.2−9.9°C northern Adriatic range, discussed above, may well be an appropriate choice. Given the more northerly location than the northern Adriatic (*c.* 45°N) it is difficult to believe that summer insolation during Ramsholt Member deposition would have been sufficient to achieve temperatures as high as those typical of the northern Adriatic (25−30°C; Fig. 5A), which are higher than the mean summer surface temperature at the more southerly (*c.* 43°N) La Franqui location (Section 2). On the other hand, even 'generous' interpretations of seafloor temperature from Ramsholt-Member shell $\delta^{18}O$ data (using a water $\delta^{18}O$ of +0.5‰; Johnson et al., 2009; Vignols et al., 2019) provide few indications of benthic temperature maxima like those in the northern Adriatic, which are rarely much below and sometimes above 20°C (Fig. 5A). The difference between annual surface and seafloor temperature maxima during Ramsholt Member deposition was therefore probably much the same as now in the northern Adriatic, and by the same token, there was probably about the same range of variation in this parameter (stratification factor).

Adopting the northern Adriatic range and favouring values in the upper part on the basis of the argument made earlier, 5, 7 and 9°C are sensible choices for stratification factors to use in calculation of a selection of surface temperature estimates for the Ramsholt Member. We need of course also to identify suitable values for water δ^{18}O, and indeed the most appropriate δ^{18}O-temperature equation. In previous work on Ramsholt-Member *A. opercularis* (Johnson et al., 2009; Vignols et al., 2019), water values of −0.5, −0.2, +0.1 and +0.5‰ were used in conjunction with the calcite equation of O'Neil et al. (1969). The first two water values are minimum and maximum estimates of the Pliocene global seawater average (Buchardt and Simonarson, 2003) and are doubtfully appropriate for a shelf basin somewhat isolated from the North Atlantic (e.g. Dearing Crampton-Flood et al., 2020, fig. 1). The last two are minimum and maximum modelled values for seawater in the Pliocene at the specific location of the Ramsholt Member (Johnson et al., 2009). The minimum modelled value yields temperatures from the co-occurring bivalve *Arctica islandica* that are all below the upper tolerance limit of modern *A. islandica* in the North Sea (16°C; Witbaard and Bergman, 2003), while the maximum modelled value yields a temperature above this from one shell (Vignols et al., 2019). Of the four values previously considered, +0.1‰ is therefore the most credible and adopted here. It is worth noting that this value is almost identical to modern seafloor values in the western part of the southern North Sea (i.e. adjacent to the location of the Ramsholt Member), and that more centrally, within the area of influence of the rivers Rhine, Meuse and Scheldt, values are only 0.2‰ lower (Harwood et al., 2008). In both the western and central southern North Sea seasonal variation in salinity is only about 0.25 PSU (Howarth et al., 1993), which translates to a seasonal variation in water δ^{18}O of just 0.07‰ using the salinity-water δ^{18}O relationship for the North Sea of Harwood et al. (2008). It seems doubtful that the additional supply of freshwater from the Baltic region in the Pliocene (e.g. Dearing Crampton-

Flood et al., 2020, fig. 1) would have had much effect on salinity, and hence water $\delta^{18}O$, at the location of the Ramsholt Member.

Table 3 about here - ? single column

We argued in Section 3.2 that the LL calcite equation of Bemis et al. (1998) is more appropriate than the calcite equation of O'Neil et al. (1969) for calculation of temperatures from *A. opercularis* shell $\delta^{18}O$. Using the former in conjunction with the minimum shell $\delta^{18}O$ values from the 10 Ramsholt-Member *A. opercularis* analysed to date, together with the water $\delta^{18}O$ value and (for surface temperatures) stratification factors identified above, yields the summer seafloor and surface temperature estimates set out in Table 3. All surface estimates with a stratification factor of 9°C are in the warm temperate summer range (20°C or above), as are six with a stratification factor of 7°C, and four with a stratification factor of 5°C. These temperatures are consistent with the dinoflagellate evidence referred to above. Included in Table 3 are winter seafloor temperatures calculated from maximum shell $\delta^{18}O$—all well within the cool temperate winter range (below 10°C). Contrary to earlier speculation (Vignols et al., 2019) it is unlikely that surface temperatures were lower, since at present in the North Sea winter seafloor and surface temperatures are identical (Fig. 1). Nevertheless, these firmly cool temperate estimates for winter, combined with the warm temperate surface estimates for summer, indicate that during Ramsholt Member deposition seasonal variation in surface temperature (perhaps > 15°C) was higher than at present in the adjacent southern North Sea (12.4°C; Fig. 1A) and much higher than in the central North Sea (7.5°C; Fig. 1B). As it is not significantly affected by assumptions about water $\delta^{18}O$, this is a robust inference, with implications in its own right for the early Pliocene climatology of NW Europe—e.g. possibly reduced oceanic heat supply (Johnson, 2009; Vignols et al., 2019). It would be desirable, however, to obtain firm estimates of absolute seasonal temperatures. The carbonate clumped isotope (Δ_{47}) technique provides a means of determining water $\delta^{18}O$ and hence of translating shell $\delta^{18}O$ values into reliable temperatures (e.g. Winkelstern et al., 2017; de Winter et al., 2018; Peral et al., 2020). Application of the technique to early and also late Pliocene marine shells from NW Europe that have already supplied $\delta^{18}O$ data (Johnson et al. 2009; Valentine et al., 2011; Vignols et al., 2019) should serve to settle current conflicts over absolute seasonal temperatures in the marine realm and enable accurate comparison with terrestrial absolute temperatures, which are presently at odds with marine temperatures over some intervals (Dearing Crampton-Flood, 2018, 2020). Such an improvement in the marine database would also assist evaluation of the roles of oceanic heat supply and radiative heating in determining regional climate.

7. Conclusions

Modern *A. opercularis* individuals from (warm temperate) sub-thermocline settings are characterised by high-amplitude variation in microgrowth-increment size over the course of ontogeny; those from supra- (and intra-) thermocline settings show much less variation. In neither sub- nor supra-thermocline settings is there a characteristic pattern of ontogenetic variation in $\delta^{13}C$ relative to $\delta^{18}O$. Shell $\delta^{18}O$ affords a fairly accurate record of winter temperature in both settings and of summer benthic temperature in supra-thermocline settings. It affords a fairly accurate record of summer benthic temperature in sub-thermocline settings during relatively cool years, but in relatively warm years temperature may be seriously underestimated.

On the basis of the findings from modern *A. opercularis*, early Pliocene specimens from the Ramsholt Member of the Coralline Crag Formation (UK) can be interpreted as sub-thermocline individuals by the evidence of their increment patterns. Subject to the accuracy of estimates of water $\delta^{18}O$ and (for summer) the difference between maximum seafloor and surface temperatures, their shell $\delta^{18}O$ indicates winter surface temperatures within the cool temperate range and summer surface temperatures at least sometimes in the warm temperate range. There can be little doubt that the seasonal range in surface temperature was higher than now in the area.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/……

References

Arthur, M.A., Williams, D.F., Jones, D.S., 1983. Seasonal temperature-salinity changes and thermocline development in the mid-Atlantic Bight as recorded by the isotopic composition of bivalves. Geol. 11, 655–659. https://doi.org/10.1130/0091-7613(1983)11<655:STCATD>2.0.CO;2.

Austin, W.E.N., Cage, A.G., Scourse, J.D. 2006. Mid-latitude shelf seas: a NW European perspective on the seasonal dynamics of temperature, salinity and oxygen isotopes. The Holocene 16, 937–947. https://doi.org/10.1177/0959683606hl985rp.

Bemis, B.E., Spero, H.J., Bijma, J., Lea, D.W., 1998. Reevaluation of the oxygen isotopic composition of planktonic foraminifera: Experimental results and revised paleotemperature equations. Paleoceanogr. 13, 150–160. https://doi.org/10.1029/98PA00070.

Broom, M.J., Mason, J., 1978. Growth and spawning in the pectinid *Chlamys opercularis* in relation to temperature and phytoplankton concentration. Mar. Biol. 47, 277–285. https://doi.org/10.1007/BF00541005.

Buchardt, B., Simonarson, L.A., 2003. Isotopic palaeotemperatiures from the Tjörnes Beds in Iceland: evidence of Pliocene cooling. Palaeogeogr. Palaeoclimatol. Palaeoecol. 189, 71–95. https://doi.org/10.1016/S0031-0182(02)00594-1.

Chauvaud, L., Thébault, J., Clavier, J., Lorrain, A., Strand, Ø., 2011. What's hiding behind ontogenetic δ^{13}C variations in mollusk shells? New Insights from the Great Scallop (*Pecten maximus*). Estuaries and Coasts 34, 20111–220. https://doi.org/10.1007/s12237-010-9267-4.

Chavanne, C., Janeković, I., Flament, P., Poulain, P.-M., Kuzmić, M., Gurgel, K.-W., 2007. Tidal currents in the northwestern Adriatic: High-frequency radio observations and numerical model predictions. J. Geophys. Res.—Oceans 112, eC03S21. https://doi.org/10.1029/2006JC003523.

Dearing Crampton-Flood, E., Peterse, F., Munsterman, D., Sinninghe Damsté, J. S., 2018. Using tetraether lipids archived in North Sea Basin sediments to extract North Western European

Pliocene continental air temperatures. Earth Planet. Sci. Lett. 490, 193–205. https://doi.org/10.1016/j.epsl.2018.03.030.

Dearing Crampton-Flood, E., Noorbergen, L.J., Smits, D., Boschman, R.C., Donders, T.H., Muns, D.K., 2020. A new age model for the Pliocene of the southern North Sea basin: a multi-proxy climate reconstruction. Clim. Past 16, 523–541. https://doi.org/10.5194/cp-16-523-2020.

De Winter, N.J., Vellekoop, J., Vorsselmans, R., Golreihan, A., Soete, J., Petersen, S.V., Meyer, K.W., Casadio, S., Speijer, R.P., Claeys, P., 2018. An assessment of latest Cretaceous *Pycnodonte vesicularis* (Lamarck, 1806) shells as records for palaeoseasonality: a multi-proxy investigation. Clim. Past 14, 725–749. https://doi.org/10.5194/cp-14-725-2018.

Djakovac, T., Supić, N., Bernardi Aubry, F., Degobbis, D., Giani, M., 2015. Mechanisms of hypoxia frequency changes in the northern Adriatic Sea during the period 1972–2012. J. Mar. Syst. 141, 179–189. https://doi.org/10.1016/j.jmarsys.2014.08.001.

Elliott, A.J., Li, Z., 1991. Thermocline depths and water temperature at selected sites in the N.W. European shelf seas. Mar. Pollut. Bull., 22, 282−286. https://doi.org/10.1016/0025-326X(91)90805-3.

Foster, L.C., Allison, N., Finch, A.A., Andersson, C., Ninnemann, U.S., 2009. Controls on δ^{18}O and δ^{13}C profiles within the aragonite bivalve *Arctica islandica*. The Holocene 19, 549–558, https://doi.org/10.1177/0959683609104028.

Gillikin, D.P., Wanamaker, A.D., Andrus, F.T., 2019. Chemical sclerochronology. Chem. Geol. 526, 1–6. https://doi.org/10.1016/j.chemgeo.2019.06.016.

Global Sea Temperature, 2020. Brighton sea temperature. https://www.seatemperature.org/europe/united-kingdom/brighton.htm (accessed 26 March 2020).

Gobler, C.J., Baumann H., 2016. Hypoxia and acidification in ocean ecosystems: coupled dynamics and effects on marine life. Biol. Lett. 12, e20150976. https://doi.org/10.1098/rsbl.2015.0976.

Gobler, C.J., DePasquale, E.L., Griffith, A.W., Baumann, H., 2014. Hypoxia and acidification have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves. PLoS ONE 9, e83648. https://doi.org/10.1371/journal.pone.0083648.

Gonfiantini, R., Stichler, W., Rozanski,, K. 1995 Standards and intercomparison materials distributed by the International Atomic Energy Agency for stable isotope measurements, in: International Atomic Energy Agency, Reference and Intercomparison Materials for Stable Isotopes of Light Elements: IAEA-TECDOC-825, Vienna, Austria, pp. 13–29.

Harwood, A.J.P., Dennis, P.F., Marca, A.D., Pilling, G.M., Millner, R.S., 2008. The oxygen isotope composition of water masses within the North Sea. Estuar. Coast. Shelf Sci. 78, 353-359. https://doi.org/10.1016/j.ecss.2007.12.010.

Head, M.J., 1997. Thermophilic dinoflagellate assemblages from the Mid-Pliocene of eastern England. J. Paleontol. 71, 165–193. https://doi.org/10.1017/S0022336000039123.

Head, M.J., 1998. New goniodomacean dinoflagellates with a compound hypotractal archeopyle from the late Cenozoic: Capisocysta Warny and Wrenn, emend. J. Paleontol. 72, 797–809. https://doi.org/10.1017/S0022336000027153.

Heilmayer, O., Brey, T., Storch, D., Mackensen, A., Arntz, W.E., 2004. Population dynamics and metabolism of *Aequipecten opercularis* (L.) from the western English Channel (Roscoff, France). J. Sea Res. 52, 33–44. https://doi.org/10.1016/j.seares.2003.07.005.

Hickson, J.A., Johnson, A.L.A., Heaton, T.H.E., Balson, P.S., 1999. The shell of the Queen Scallop *Aequipecten opercularis* (L.) as a promising tool for palaeoenvironmental reconstruction: evidence and reasons for equilibrium stable-isotope incorporation. Palaeogeogr. Palaeoclimatol. Palaeoecol. 154, 325–337. https://doi.org/10.1016/S0031-0182(99)00120-0.

Hickson, J.A., Johnson, A.L.A., Heaton, T.H.E., Balson, P.S., 2000. Late Holocene environment of the southern North Sea from the stable isotopic composition of Queen Scallop shells.

Palaeontolog. Electron. 3, iss. 2, art. 3, 11 pp. http://palaeo-electronica.org/2000_2/scallop/issue2_00.htm.

Howarth, M.J., Dyer, K.R., Joint, I.R., Hydes, D.J., Purdie, D.A., Edmunds, H., Jones, J.E., Lowry, R.K., Moffat, T.J., Pomroy, A.J., Proctor, R. 1993. Seasonal cycles and their variability. Philosophical Transactions R. Soc. A, 343, 383−403. https://doi.org/10.1098/rsta.1993.0054.

Janeković, I., Dutour Sikirić, M., Tomažić, I., Kuzmić, M., 2010. Hindcasting the Adriatic Sea surface temperature and salinity: A recent modeling experience. Geofizika 27, 85–100.

Janeković, I., Mihanović, H., Vilibić, I., Tudor, M., 2014. Extreme cooling and dense water formation estimates in open and coastal regions of the Adriatic Sea during the winter of 2012. J. Geophys. Res.—Oceans 119, 3200–3218. https://doi.org/10.1002/2014JC009865.

Johnson, A.L.A., Hickson, J.A., Bird, A., Schöne, B.R., Balson, P.S., Heaton, T.H.E., Williams, M., 2009. Comparative sclerochronology of modern and mid-Pliocene (c. 3.5 Ma) *Aequipecten opercularis* (Mollusca, Bivalvia): an insight into past and future climate change in the north-east Atlantic region. Palaeogeogr. Palaeoclimatol. Palaeoecol. 284, 164–179. https://doi.org/10.1016/j.palaeo.2009.09.022.

Johnson, A.L.A., Valentine, A., Leng, M.J., Sloane, H.J., Schöne, B.R., Balson, P.S., 2017. Isotopic temperatures from the Early and Mid-Pliocene of the US Middle Atlantic Coastal Plain, and their implications for the cause of regional marine climate change. Palaios 32, 250–269. https://doi.org/10.2110/palo.2016.080.

Johnson, A.L.A., Valentine, A.M., Leng, M.J., Schöne, B.R., Sloane, H.J., 2019. Life history, environment and extinction of the scallop *Carolinapecten eboreus* (Conrad) in the Plio-Pleistocene of the US eastern seaboard. Palaios 34, 49–70. https://doi.org/10.2110/palo.2018.056.

Kim, S.-T., O'Neil, J.R., 1997. Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. Geochim. Cosmochim. Acta 61, 3461–3475. https://doi.org/10.1016/S0016-7037(97)00169-5.

Kralj, M., Lipizer, M., Čermelj, B., Celio, M., Fabbro, C., Brunetti, F., Francé, J., Mozetič, P., Giani, M., 2019. Hypoxia and dissolved oxygen trends in the northeastern Adriatic Sea (Gulf of Trieste). Deep Sea Res. Part II: Topical Stud. Oceanography, 164, 74−88. https://doi.org/10.1016/j.dsr2.2019.06.002.

Krantz, D.E., 1990. Mollusk-isotope records of Plio-Pleistocene marine paleoclimate, U.S. Middle Atlantic Coastal Plain. Palaios 5, 317–335. https://doi.org/10.2307/3514888.

Krantz, D.E., Jones, D.S., Williams, D.F., 1984. Growth rates of the sea scallop, *Placopecten magellanicus*, determined from the $^{18}O/^{16}O$ record in shell calcite. Biol. Bull. 167, 186–199. https://doi.org/10.2307/1541347.

Krantz, D.E., Williams, D.F., Jones, D.S., 1987. Ecological and paleoenvironmental information using stable isotope profiles from living and fossil molluscs: Palaeogeogr. Palaeoclimatol. Palaeoecol. 58, 249–266. https://doi.org/10.1016/0031-0182(87)90064-2.

Long, P.E., Zalasiewicz, J.A., 2011. The molluscan fauna of the Coralline Crag (Pliocene, Zanclean) at Raydon Hall, Suffolk, UK: Palaeoecological significance reassessed. Palaeogeogr. Palaeoclimatol. Palaeoecol. 309, 53–72. https://doi.org/10.1016/j.palaeo.2011.05.039.

Lorrain, A., Paulet, Y.M., Chauvaud, L., Dunbar, R., Mucciarone, D., Fontugne, M., 2004. $\delta^{13}C$ variation in scallop shells: Increasing metabolic carbon contribution with body size? Geochim.Cosmochim. Acta 68, 3509–3519. https://doi.org/10.1016/j.gca.2004.01.025.

Marchais, V., Richard, J., Jolivet, A., Flye-Sainte-Marie, J., Thébault, J., Jean, F., Richard, P., Paulet, Y.-M., Clavier, J., Chauvaud, L., 2015. Coupling experimental and field-based approaches to decipher carbon sources in the shell of the great scallop, *Pecten maximus* (L.). Geoch. Cosmochim. Acta 168, 58–69. https://doi.org/10.1016/j.gca.2015.07.010.

McConnaughey, T.A., Gillikin, D.P., 2008. Carbon isotopes in mollusk shell carbonates. Geo-Mar. Lett. 28, 287–299. https://doi.org/10.1007/s00367-008-0116-4.

Mook, W.G., Vogel, J.C., 1968. Isotopic equilibrium between shells and their environment. Science 159, 874–875. https://doi.org/10.1126/science.159.3817.874.

NOAA (US Department of Commerce, National Oceanic and Atmospheric Administration, 1994. NODC (Levitus) World Ocean Atlas: Ocean Temperature: Monthly Long Term Mean. https://www.esrl.noaa.gov/psd/cgi-bin/db_search/DBSearch.pl?Dataset=NODC+(Levitus)+World+Ocean+Atlas&Variable=Ocean+temperature (accessed 25 March 2020).

O'Neil, J.R., Clayton, R.N., Mayeda, T.K., 1969. Oxygen isotope fractionation in divalent metal carbonates. J. Chem. Phys. 51, 5547–5558. https://doi.org/10.1063/1.1671982.

Owen, R., Richardson, C., Kennedy, H., 2002. The influence of shell growth rate on striae deposition in the scallop *Pecten maximus.* J. Mar. Biol. Assoc. U. K. 82, 621–623. https://doi.org/10.1017/S0025315402005969.

Peharda, M., Thébault, J., Markulin, K., Schöne, B.R., Janeković, I., Chauvaud, L., 2019. Contrasting shell growth strategies in two Mediterranean bivalves revealed by oxygen-isotope ratio geochemistry: The case of *Pecten jacobaeus* and *Glycymeris pilosa*. Chem. Geol. 526, 23–35. https://doi.org/10.1016/j.chemgeo.2017.09.029.

Peral, M., Blamart, D., Bassinot, F., Daëron, M., Dewilde, F., Rebaubier, H, Nomade, S., Girone, A, Marino, M., Maiorano, P, Ciaranfi, N., 2020. Changes in temperature and oxygen isotopic composition of Mediterranean water during the Mid-Pleistocene transition in the Montalbano Jonico section (southern Italy) using the clumped-isotope thermometer. Palaeogeogr. Palaeoclimatol. Palaeoecol. 544, https://doi.org/10.1016/j.palaeo.2020.109603.

Pierre, C., 1999. The oxygen and carbon isotope distribution in the Mediterranean water masses. Mar. Geol. 153, 41–55. https://doi.org/10.1016/S0025-3227(98)00090-5.

Prendergast, A.L., Versteegh, E.A.A., Schöne, B.R., 2017. New research on the development of high-resolution palaeoenvironmental proxies from geochemical properties of biogenic carbonates. Palaeogeogr. Palaeoclimatol. Palaeoecol. 484, 1–6. https://doi.org/10.1016/j.palaeo.2017.05.032.

Ren, J.S., Ross, A.H.. Schiel, D.R., 2000. Functional descriptions of feeding and energetics of the Pacific oyster *Crassostrea gigas* in New Zealand. Mar. Ecol., Prog. Series 208, 119–130. https://doi.org/10.3354/meps208119.

Schöne, B.R., Surge, D., 2005. Looking back over skeletal diaries — High-resolution environmental reconstructions from accretionary hard parts of aquatic organisms. Palaeogeogr. Palaeoclimatol. Palaeoecol. 228, 1–3. https://doi.org/10.1016/j.palaeo.2005.03.043.

Schöne, B.R., Fiebig, J., 2009. Seasonality in the North Sea during the Allerød and Late Medieval Climate Optimum using bivalve sclerochronology. Int. J. Earth Sci. 98, 83–98. https://doi.org/10.1007/s00531-008-0363-7.

Schöne, B.R., Gillikin, D.P., 2013. Unravelling environmental histories from skeletal diaries—Advances in sclerochronology. Palaeogeogr. Palaeoclimatol. Palaeoecol. 373, 1–5. https://doi.org/10.1016/j.palaeo.2012.11.026.

Schöne, B.R., Freyre Castro, A.D., Fiebig, J., Houk, S.D., Oschmann, W., Kröncke, I., 2004. Sea surface water temperatures over the period 1884–1983 reconstructed from oxygen isotope ratios of a bivalve mollusc shell (*Arctica islandica*, southern North Sea). Palaeogeogr. Palaeoclimatol. Palaeoecol. 212, 215–232. https://doi.org/10.1016/j.palaeo.2004.05.024.

Schöne, B.R., Houk, S.D., Freyre Castro, A.D., Fiebig, J., Kröncke, I., Dreyer, W., Oschmann, W., 2005a. Daily growth rates in shells of *Arctica islandica*: assessing subseasonal environmental controls on a long-lived bivalve mollusk. Palaios 20, 78–92. https://doi.org/10.2110/palo.2003.p03-101.

Schöne, B.R., Fiebig, J., Pfeiffer, M., Gleß, R., Hickson, J., Johnson, A.L.A., Dreyer, W., Oschmann, W., 2005b. Climate records from a bivalved Methuselah (*Arctica islandica*, Mollusca; Iceland). Palaeogeogr. Palaeoclimatol. Palaeoecol. 228, 130–148. https://doi.org/10.1016/j.palaeo.2005.03.049.

Schöne, B.R., Schmitt, K., Maus, M., 2017. Effects of sample pretreatment and external contamination on bivalve shell and Carrara marble $\delta^{18}O$ and $\delta^{13}C$ signatures. Palaeogeogr. Palaeoclimatol. Palaeoecol. 484, 22–32. https://doi.org/10.1016/j.palaeo.2016.10.026.

Stenni, B., Nichetto, P., Bregant, D., Scarazzato, P., Longinelli, A., 1995. The $\delta^{18}O$ signal of the northward flow of Mediterranean waters in the Adriatic Sea. Oceanol. Acta 18, 319–328.

Taylor, A.C., Venn, T.J., 1978. Growth of the queen scallop, *Chlamys opercularis*, from the Clyde Sea area. J. Mar. Biol. Assoc. U. K. 58, 687–700. https://doi.org/10.1017/S0025315400041333.

Valentine, A., Johnson, A.L.A., Leng, M.J., Sloane, H.J., Balson, P.S., 2011. Isotopic evidence of cool winter conditions in the mid-Piacenzian (Pliocene) of the southern North Sea Basin. Palaeogeogr. Palaeoclimatol. Palaeoecol. 309, 9–16. https://doi.org/10.1016/j.palaeo.2011.05.015.

van Leeuwen, S., Tett, P., Mills, D., van der Molen, J., 2015. Stratified and nonstratified areas in the North Sea: Long-term variability and biological and policy implications, J. Geophys. Res.—Oceans 120, 4670–4686. https://doi.org/10.1002/2014JC010485.

Vignols, R.M., Valentine, A.M., Finlayson, A.G., Harper, E.M., Schöne, B.R., Leng, M.J., Sloane, H.J., Johnson, A.L.A., 2019. Marine climate and hydrography of the Coralline Crag (early Pliocene, UK): isotopic evidence from 16 benthic invertebrate taxa. Chem. Geol. 536, 62–83. https://doi.org/doi:10.1016/j.chemgeo.2018.05.034.

VisitMyHarbour, 2012. Hourly tidal streams, English Channel East. https://www.visitmyharbour.com/articles/3173/hourly-tidal-streams-english-channel-east/ (accessed 26 March 2020).

Vilibić, I., Mihanović, H., Janeković, I., Šepić, J., 2016. Modelling the formation of dense water in the northern Adriatic: Sensitivity studies. Ocean Model. 101, 17–29. https:doi.org/10.1016/j.ocemod.2016.03.001.

Winkelstern, I. Z., Rowe, M.P., Lohmann, K.C., Defliese, W.F., Petersen, S.V., Brewer , A.W., 2017. Meltwater pulse recorded in Last Interglacial mollusk shells from Bermuda. Paleoceanogr. 32. https://doi.org/10.1002/2016PA003014.

Witbaard, R., Bergman, M.J.N., 2003. The distribution and population structure of the bivalve *Arctica islandica* L. in the North Sea: what possible factors are involved? J. Sea Res. 50, 11–25. https://doi.org/10.1016/S1385-1101(03)00039-X.

FIGURE CAPTIONS

Fig. 1. Profiles of monthly average surface (red line) and seafloor (blue line; red where identical with surface) temperature for locations in the southern (A) and central (B) North Sea (data from Austin et al., 2006, fig. 8). Note the almost identical summer surface and seafloor temperatures at the shallow (well-mixed) southern location and the strongly divergent summer temperatures at the deeper (seasonally stratified) northern location (see also Elliott and Li, 1991; van Leeuwen et al., 2015). Summer and winter surface temperatures are within the respective cool temperate ranges (< 20°C, < 10°C; Vignols et al., 2019) at both locations.

Fig. 2. Profiles of ontogenetic variation in $\delta^{13}C$ (grey), $\delta^{18}O$ (pink) and microgrowth-increment height (green; thicker, continuous line connects 5-point moving averages) in *Aequipecten opercularis* from:

(A) a supra-thermocline setting in the UK sector of the southern North Sea (macrotidal); (B) a sub-thermocline setting in the Gulf of Tunis, southern Mediterranean Sea (microtidal, 50 m depth); (C) the Ramsholt Member of the Coralline Crag Formation (early Pliocene), Suffolk, eastern England. Specimens represented are, respectively, British Geological Survey (BGS) Zt 9957, Muséum National d'Histoire Naturelle, Paris (MNHN), IM-2008-1537, and University of Derby, Geological Collections (UD) 52795 (illustrated in Fig. 3A). Data from Johnson et al. (2009), where further background information can be found. Specific location for BGS Zt 9957 given in Hickson et al. (2000); general location for all shells shown in Fig. 4. The isotopic axis has been reversed in each part so that lower values of shell $\delta^{18}O$ (corresponding to higher temperatures) plot towards the top. Note that data for BGS Zt 9957, a specimen from which the dorsal part had been broken off, are here correctly plotted (as by Hickson et al., 2000, fig. 4) in relation to height from the ventral margin, unlike in plots by Johnson et al. (2009, fig 4D; 2017, fig. 5B). Open triangles indicate the position of minor growth breaks. Value shown for variation in increment height is the difference between the maximum and minimum of smoothed (5-point moving average) data.

Fig. 3. Microgrowth increments in *Aequipecten opercularis.* A: the early Pliocene specimen (right valve) from which the measurements in Fig. 2C were obtained; B: the modern Adriatic specimen S3A4 (left valve) from which the measurements in Fig. 7C were obtained; C: the modern English Channel specimen EC1 (left valve) from which the measurements in Fig. 8A were obtained. Note the substantial variation in increment size in A and B, and the much more uniform increment size in C. Triangles indicate the positions of minor (open) and moderate–major (filled) growth breaks within the area of the enlargements. Scale bars = 10 mm; vertical for full-shell images, horizontal for enlargements. Microgrowth increments have been described as 'striae' in work on this and other scallop species (e.g. Broom and Mason, 1978; Owen et al., 2002; Peharda et al., 2019). They are bounded by commarginal lamellae, which on left valves (e.g. B, C) are commonly discontinuous

between the plicae (‘ribs’), making it difficult to define increments there. In such circumstances measurements were made on the plicae.

Fig. 4. Collection locations of the Adriatic (1), English Channel (2) and French Mediterranean (3) shells, and of the shells represented in Fig. 2A (4), Fig. 2B (5) and Fig. 2C (6). See Section 2 and Table 1 for details.

Fig. 5. A: Profiles of daily temperature from 2008–2016 for the surface (red line) and seafloor (38 m depth; blue line) at the location of the Adriatic specimens, derived using the numerical ocean model ROMS. B: Profiles of daily salinity and water δ^{18}O at the seafloor for the same location and interval; salinity derived using ROMS and water δ^{18}O derived using the salinity-water δ^{18}O relationship of Peharda et al. (2019), based on the data of Stenni et al. (1995) from adjacent sites in the northern Adriatic. C: Predicted shell (calcite) δ^{18}O for the location and depth of the Adriatic specimens, derived using the seafloor temperature and water δ^{18}O data in A and B, and the LL equation of Bemis et al. (1998). Note that the δ^{18}O axis has been reversed in C (see Fig. 2 for explanation). Error estimates have been excluded for the sake of clarity but are included in the shorter profile of predicted shell δ^{18}O (2013–2016) in Fig. 10. All data available online (see Appendix A).

Fig. 6. Shell δ^{18}O values (filled circles) from Fig. 2A (supra-thermocline *A. opercularis* from 53°N in the North Sea) temporally aligned with a curve (pink line) of expected monthly shell (calcite) δ^{18}O for 53°N in the North Sea, derived using the LL equation of Bemis et al. (1998) and the monthly average seafloor temperature and water δ^{18}O data of Austin et al. (2006, fig. 8), replicated over four years (note that the δ^{18}O axis has been reversed; see Fig. 2 for explanation). Also included are curves based on the same data but derived using the calcite equations of O’Neil et al. (1969; lavender) and Kim and O’Neil (1997; gold). Minor growth breaks (open triangles) inserted in accordance with their position

relative to measured $\delta^{18}O$ values (Fig. 2A). The decreasing completeness of the measured record over time probably reflects increasing time-averaging within samples due to ontogenetic decline in growth rate.

Fig. 7. Isotope and increment data for Adriatic shells S3A1 (A), S3A3 (B), S3A4 (C), S3A5 (D), S3A33 (E) and S3A36 (F), plotted as in Fig. 2, with a pale green background used to correspond with Fig. 2B (data also from a sub-thermocline setting). Isotope profiles link singleton or mean values (crosses indicate the values from which these were derived). Open and filled triangles indicate the position of minor and moderate–major growth breaks, respectively. S3A4 is illustrated in Fig. 3B.

Fig. 8. Isotope and increment data for English Channel shells EC1 (A) and EC2 (B), plotted as in Fig. 2, with a pale blue background used to correspond with Fig. 2A (data also from a supra-thermocline setting). Isotope profiles link singleton or mean values (crosses indicate the values from which these were derived). Open and filled triangles indicate the position of minor and moderate–major growth breaks, respectively. EC1 is illustrated in Fig. 3C.

Fig. 9. Isotope and increment data for French Mediterranean shells FM1 (A) and FM2 (B), plotted as in Fig. 2, with a pale blue background used to correspond with Fig. 2A (data also from a supra-thermocline setting). Increment data from 55–60 mm height in FM2 have been excluded in the interests of clarity and comparability with other datasets; inclusion of these data (available online; see Appendix A) would not have increased the range of variation recorded. Open and filled triangles indicate the position of minor and moderate–major growth breaks, respectively.

Fig. 10. Values of $\delta^{18}O$ (circles; open for anomalous values) from Adriatic shells S3A1 (A), S3A3 (B), S3A4 (C), S3A5 (D), S3A33 (E) and S3A36 (F), aligned with a curve of predicted daily values.

Note that the $\delta^{18}O$ axis has been reversed (see Fig. 2 for explanation). Measured values from Fig. 7 (individual values contributing to means excluded); predicted values from Fig. 5C, with added error estimates (dotted lines) based on 1σ values for modelled temperature and salinity. Minor and moderate–major growth breaks (open and filled triangles, respectively) inserted in accordance with their position relative to measured $\delta^{18}O$ values (Fig. 7). Vertical grey bars signify gaps of > 100 days in the sequence of measured $\delta^{18}O$ values. References in the text to winters in specific calendar years refer to the cold period (i.e. the interval of high $\delta^{18}O$) at the start of the year stated.

Fig. 11. Scatterplot showing the relatively low $\delta^{13}C$ values from Adriatic shells (open orange diamonds), the relatively high values from English Channel shells (filled teal diamonds), and the intermediate values from French Mediterranean shells (black crosses), independent of $\delta^{18}O$. Data from the profiles in Figs 7–9.

TABLE CAPTIONS

Table 1

Locational information and descriptive statistics for shell $\delta^{13}C$ and $\delta^{18}O$

Table 2

Modelled annual surface and seafloor temperature maxima in the northern Adriatic (Fig. 5A; Appendix A), and inferred surface maxima from accurate and underestimated (2015, 2016) seafloor data, using various stratification factors (see text for explanation of underestimation and stratification factors)

Table 3

Seasonal seafloor and surface temperatures from $\delta^{18}O$ of Ramsholt Member *A. opercularis*, calculated using the LL equation of Bemis et al. (1998) and water $\delta^{18}O$ of +0.1‰

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