Tropical seasonality in the late Campanian (late Cretaceous): Comparison between multiproxy records from three bivalve taxa from Oman

Niels J. de Winter, Steven Goderis, Frank Dehairs, John W.M. Jagt, René H.B. Fraaije, Stijn J.M. Van Malderen, Frank Vanhaecke, Philippe Claeyts

1. Introduction

1.1. Late cretaceous climate reconstruction

The Late Cretaceous is a well-studied interval of geological time, in part due to its hothouse climate punctuated by episodes of rapid climatic and environmental change (Jenkyns, 1980; Huber et al., 1995; Li and Keller, 1999; Jenkyns et al., 2004). During the Late Cretaceous, atmospheric pCO2 levels are estimated to have been two to four times higher (i.e. ~400 to ~1000 ppmV) compared to the pre-industrial level (280 ppmV; e.g., Berner, 1990; Andrews et al., 1995; Ekart et al., 1999; Quan et al., 2009). The values projected by the Intergovernmental Panel on Climate Change (IPCC, 2013) indicate that such high values may be reached within the 21st century (280 ppmV; e.g., Dlugokencky and Tans, 2017). This makes the Late Cretaceous an important geological time period to study the effects of increased pCO2 on Earth’s climate. As such, long-term changes in climate and environment during this time period have received much attention (e.g.,...
1.2. Bivalve shells as climate recorders

Fossil bivalve shells are well-studied records that track these sub-annual climate fluctuations. Marine bivalves record changes in their environment, and thus local climate, as a result of environment-dependent chemical and isotopic fractionation that takes place during the incremental growth of their carbonate (aragonitic/calcitic) shell (Epstein et al., 1953; Grossman and Ku, 1986; Jones, 1980, 1983; Klein et al., 1996a; Lazareth et al., 2003). Chemical variations of pristinely preserved shell material can record changes in the animal's environment and local climate down to daily resolution, much higher than can be retrieved through conventional long timescale climate archives. Thick-shelled rudistid (Mollusca: Haploplacida, see Newell, 1965) and ostreid bivalves (Mollusca: Ostreoida, see Bouchez et al., 2010) are suitable taxa for this type of research because their thick calcitic shells are more resistant to diagenesis than those of their aragonitic relatives (Al-Aasm and Veizer, 1986a, 1986b; Steuber, 1999; Ullmann and Korte, 2015). The use of such shells to reconstruct climate on a sub-seasonal scale has been amply demonstrated by earlier work on both rudistid (Steuber, 1996, 1999; Steuber et al., 2005; Immenhauser et al., 2005; Hennhöfer et al., 2012) and ostreid shells (Kirby et al., 1998; Brigaud et al., 2008; Ullmann et al., 2010; Fan et al., 2011). However, there are complications with the use of shells of extinct bivalve species to reconstruct climate because of potential species-specific differences in physiological (“vital”) effects, such as the effects of varying growth and metabolic rate on palaeoclimatic proxies (Fastovsky et al., 1993; Steuber, 1999; Lorrain et al., 2005; Gillikin et al., 2005; Immenhauser et al., 2005; Schöne et al., 2011). This requires the construction of species-specific transfer functions for the relationship between climatic and environmental parameters and geochemical proxies in the shell, which requires culture experiments (e.g. Wannamaker et al., 2007; Freitas et al., 2008). For extinct bivalve species, proxy data sets can be cross calibrated using related extant species (e.g. Armendáriz et al., 2008). For species without close extant relatives, such as rudistids, the use of modern bivalve transfer functions can, however, severely reduce the reliability of reconstructions.

1.3. Multi-species approach

The reliability of proxy interpretation in these bivalves can be improved by comparing records from multiple species and by using multiple proxies. This approach allows isolation of the effect of different parameters on the measured proxies when culture experiments cannot be carried out (Klein et al., 1996b; Carroll et al., 2009). The present work discusses a multi-proxy analysis of a single ostreid shell and two associated rudistid taxa from the Campanian Samhan Formation from the Saiwan area in the Sultanaate of Oman, which is characterized by long horizontally-continuous successions of reef-like deposits of bivalves, stromatoporoids and corals. This shallow marine Tethys Ocean succession contains exceptionally well-preserved rudistids and other bivalves in life position, allowing multi-species palaeoenvironmental reconstructions to be placed in a wider framework of Late Cretaceous reconstructions in the Tethys Ocean. The use of three different species in a multi-proxy approach enables an assessment of potential species-specific differences in proxy relationships and offers a unique opportunity to isolate seasonal fluctuations of climatic parameters recorded in extinct bivalve species. In addition to reconstructing past seasonality, the use of multiple species and multiple proxies permits a more general discussion of the value of trace element and stable isotope records from fossil bivalve shells.

2. Background

2.1. Palaeoenvironmental proxies in calcite skeletons

A wide range of marine calcifying organisms has been the subject of studies aiming to reconstruct sub-annual climate. Detailed studies of stable isotope ratios and trace element concentrations in corals (Goreau, 1977; Swart, 1983; Sinclair et al., 1998), coralline algae (Halfar et al., 2000; Hetzinger et al., 2011), scleractinians (Lazareth et al., 2000; Swart et al., 2002), molluscs (e.g. Jacob et al., 2008; McConnaughey and Gillikin, 2008; Elliott et al., 2009; Goodwin et al., 2013; Ullmann et al., 2010, 2013, 2015), brachiopods (Veizer et al., 1986; Grossman et al., 1996) and stromatolites (Chafetz et al., 1991; Andrews and Brasier, 2005) have shown that many of these organisms and colonies record changes in the chemistry of their carbonate skeleton in response to seasonal changes in their environment. It is reported that most of these marine organisms precipitate their carbonate skeletons in equilibrium with ambient seawater, allowing sub-annual reconstruction of palaeotemperature from their stable isotope ratios. However, several other environmental parameters, such as salinity, nutrient availability and sea water chemistry, complicate the reconstruction of palaeoseasonality in shallow marine records (Klein et al., 1996b; Purton and Brasier, 1997; Surge and Lohmann, 2002). In response to these complications and in an attempt to obtain a more complete picture of sub-annual variations in the local environment, several trace element proxies for changes in sea water chemistry have been developed for calcifying organisms. Magnesium-to-calcium (Mg/Ca) ratios in carbonates have been proposed as proxies for palaeotemperature, potentially allowing independent control on temperature reconstructions and the determination of salinity changes in combination with stable oxygen isotope ratios (Mitsuguchi et al., 1996; Klein et al., 1996a; Richardson et al., 2004; Wannamaker et al., 2008; Schöne et al., 2011). Additionally, strontium-to-calcium (Sr/Ca) ratios have been shown to vary with calcification temperature and salinity in some bivalves and corals (Dodd and Crisp, 1982; de Villiers et al., 1995; Richardson et al., 2004; Freitas et al., 2006). Several trace element proxies have been suggested to record changes in palaeoproductivity and redox conditions in ambient sea water. Examples include redox sensitive elements such as manganese (Mn; Freitas et al., 2006), elements that are enriched in the skeletons of primary producers such as barium (Ba; Gillikin et al., 2008; Malari and Schöne, 2015) and micronutrients such as zinc (Zn) and cadmium (Cd) which are readily taken up into the shells of bivalves (Carriker et al., 1980; Calmano et al., 1993; Jackson et al., 1993; Wang and Fisher, 1996; Guo et al., 1997). Other studies have demonstrated the reproducibility of trace element profiles between specimens living in the same environment, showing that these records are likely to be governed by environmental factors (Gillikin et al., 2008; Malari et al., 2017).

2.2. Vital effects

However, it is now recognized that trace elements are often not taken up in equilibrium with the environment and that vital effects influence many of these proxies (see Weiner and Dove, 2003 and references therein). Examples include studies that show relationships between trace element concentrations and mineral growth in bivalves (Lorrain et al., 2005; Gillikin et al., 2005; Freitas et al., 2006; Carré et al., 2006; Schöne et al., 2011), evidence of the breakdown of proxy relationships during periods of growth stress (Lorens and Bender, 1980; Hendry et al., 2001; Takesue and van Geen, 2004) and the effect of
changing redox conditions in the sea water (Vander Putten et al., 2000; Hendry et al., 2001; Freitas et al., 2006; Beck et al., 2017). Independent chronologies and proxies for the amount of organic matrix in the shell (e.g. sulphur (S) concentrations) may be used to better constrain vital effects in biogenic carbonate records by reconstructing the physiological cycle of the organism (de Villiers et al., 1994; Sinclair et al., 1998; Rosenberg et al., 2001; Dauphin et al., 2003; Marali and Schöne, 2015). An example of how the integration of proxies for physiological changes enables the discussion of proxy interpretation, is the observation of covarying magnesium (Mg) and sulphur (S) concentrations in bivalve records as evidence for physiological control on the incorporation of trace elements into bivalve shell calcite (Lorens and Bender, 1980; Dauphin et al., 2003; England et al., 2007; Perrin et al., 2017). Recent studies have therefore focused on the integration of multi-proxy records from multiple sub-annual climate records in order to isolate the effects of environmental parameters (e.g. Black et al., 2009; Wanamaker et al., 2011; Marali et al., 2017).

2.3. Geological setting

The three bivalve specimens described in this study originate from the upper Campanian middle member of the Samhan Formation in the Saiwan area of the Huqf Desert in east-central Oman (Schumann, 1995). The two rudistid specimens were retrieved in life position from a 200 m wide Vaccinites-dominated rudist biostrome that contains many large Torreites rudists (Unit V1 in profile 1 in Schumann, 1995; rudist unit 2 in Philip and Platel, 1995), while the thick-shelled oyster (Oscillopha figari) originated from the top of the marly limestone unit directly below the rudistid horizon (top of Unit 1 in profile 1 in Schumann, 1995). The Samhan Formation records the development of a shallow marine carbonate platform during a gradual transgression (Platel et al., 1994). Previously established as early Campanian (Platel et al., 1994), the middle and upper Samhan Formation were later dated as late Campanian using ammonite biostratigraphy by Kennedy et al. (2000). This is in congruence with the dating of the Orbitoides tissoti zone, in which the Samhan Formation is found (Philip and Platel, 1995), to the early late Campanian (Korbar et al., 2010). Schumann (1995) described the Samhan Formation as a shell setting far from the coast, with no evidence of coastal sedimentation. The palaeoenvironment was frequently disturbed by strong currents and storm events and was characterized by high input of weathering products from Omani ophiolites. The Vaccinites-dominated biostromes are presumably formed during periods of relatively lower hydro-energy and were very short-lived (100 s of years; Schumann, 1995). However, it is hypothesized that the low diversity of these biostromes suggests an adaptation of Vaccinites and Torreites to a highly turbulent environment. The O. figari specimen was found 1-2 m below the biostrome and is therefore assumed to have lived before the closely associated rudistid species.

2.4. Late Campanian palaeoclimate

The late Campanian period is characterized by a climatic optimum with mean annual sea surface temperatures in northern Europe rising to 19–20 °C (Thibault et al., 2016). Modelling results indicate that mean annual temperatures were approximately 10 °C warmer compared to today (Deconto, 1996), but meridional temperature gradients were probably lower, meaning that tropical temperatures were likely less different from the present day situation, with tropical surface temperatures estimated at 30 °C (Huber et al., 1995; Brady et al., 1998; Alsenz et al., 2013). At the same time, a higher eustatic sea level caused continental shelves to be submerged and continents were likely surrounded by extensive shallow seas (Brady et al., 1998). The geographical setting of this study’s locality fits this description, with shallow marine conditions above the storm wave base occurring without signs of deltaic sedimentation. Palaeoceanographic studies in the area have demonstrated that the Late Cretaceous Tethys Ocean was prone to rapid changes in palaeoproductivity and redox conditions as the basin became progressively more confined (Dercourt et al., 1986; Almogi-Labin et al., 1993; Steuber and Löser, 2000; Jarvis et al., 2002; Fleurance et al., 2013). Besides these local changes, long-term trends in global ocean water chemistry also significantly influenced the environment in which the bivalves in this study precipitated their shells. Marine magnesium-to-calcium (Mg/Ca) and strontium-to-calcium (Sr/Ca) ratios were much lower (1–2 mol/mol and 4–6 mmol/mol, respectively, compared to 5 mol/mol and 8–9 mmol/mol in modern oceans; Stanley and Hardie, 1998; Coggon et al., 2010; Rausch et al., 2013), which is likely to have influenced the trace element concentrations recorded in shell calcite (e.g. Lear et al., 2015).

3. Methods

3.1. Specimen acquisition and preparation

The shells of two rudistid bivalves (Torreites sanchezi milovanovicorum Douville, 1927; Naturhistorisches Museum Maastricht collections, NHMM 2014 052, hereafter TS and Vaccinites vesiculosus Woodward, 1855; Oerijtijmuseum De Groene Poort collections, MAB D.R.81 5/19078, hereafter VV) and a thick-shelled oyster (Oscillopha figari Fourtou, 1904, NHMM 2014 050a, hereafter OF; Malchus, 1990; Aqrabawi, 1993) were acquired for the present study. The VV shell was subject of an earlier publication by de Winter and Claeys (2016). All shells were collected in life position in the upper Campanian Samhan Formation in the Saiwan area of the Huqf Desert in east-central Oman (Unit 1 of Schumann, 1995, 30°39’N, 57°31’E, see also Platel et al., 1994; Philip and Platel, 1995; Kennedy et al., 2000 and section above). Shells were cut longitudinally along their central growth axis and high-grade polished by increasingly fine-grained silicon carbide polishing discs (up to P2400) at the Vrije Universiteit Brussel (VUB, Belgium). High-resolution (6400 dpi) colour scans were produced of the shell surfaces to track all sample locations in an overview figure (Fig. 1).

3.2. μXRF element mapping

High-resolution elemental abundance maps of the polished surface of shells were created using a Tornado M4 micro X-ray fluorescence (μXRF) scanner (Bruker nano GmbH, Berlin, Germany). μXRF mapping was done along a 2D grid with 50 μm spacing, a spot size of 25 μm and an integration time of 1 ms per pixel. The X-ray source was operated under maximum energy settings (600 μA, 50 kV) and no source filters were applied. Details of the setup of the Tornado M4 scanner for the mapping of bivalve calcite can be found in de Winter and Claeys (2016). Higher resolution (25 μm spacing) maps were made of smaller areas of the shells. Mapping by μXRF permitted qualitative element abundance distributions to be visualized (see Fig. 1).

3.3. μXRF line scanning

To determine the best approach for μXRF scanning, trace element profiles were measured on the outer calcite layer along the length of the VV shell using three methods: 1) conventional μXRF line scanning according to de Winter and Claeys (2016), 2) a μXRF point-by-point line scanning method with longer integration time (60 s) after de Winter et al. (2017), and 3) two different transects quantified by Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS). To optimize the results, XRF maps were used to guide sampling of well-preserved parts of the shells in all transient line scanning methods. The method for conventional XRF line scanning on bivalve calcite is detailed in de Winter and Claeys (2016). For point-by-point line scanning, the X-ray beam is allowed to dwell on each point for 60 s at maximized energy settings (50 kV, 600 μA, no source filter) with a sampling resolution of 100 μm for a total of 978 and 1051 points respectively for VV and...
TS and a total of 431 points for the OF shell. This allows more time for each XRF spectrum to be accumulated and results in more reproducible results (de Winter et al., 2017). All XRF line scans were quantified according to the Fundamental Parameters method of the Esprit software calibrated to BAS CRM393 limestone trace element reference material (Bureau of Analyzed Samples Ltd., UK). XRF point-by-point scans were carried out on the outer calcite layer in the growth direction of the rudistid shells (VV and TS) and through a polished section of the hinge of the OF shell perpendicular to the growth layers (see Fig. 1). 3.4. LA-ICP-MS line scanning

LA-ICP-MS measurements were carried out at the Atomic and Mass Spectrometry research unit of Ghent University (Ghent, Belgium). A detailed account of LA-ICP-MS methodology is found in Supplementary data 1. XRF line scans were calibrated by using the bulk concentration of one of the LA-ICP-MS transects as an additional standard and establishing a calibration curve. The results of the application of these methods and a schematic overview of the calibration of XRF results with LA-ICP-MS results are shown in Fig. 2. A detailed explanation of the calibration calculations is given in Supplementary data 1. After this correction, the average offset of strontium (Sr) concentrations between corrected XRF measurements and results of the second LA-ICP-MS line scan (not used in the correction) was 9.1% for point-by-point analysis and 12% for conventional line scan analysis. All trace element profiles discussed further in this study are measured by point-by-point XRF line scanning and calibrated using the LA-ICP-MS calibration. Reproducibility standard deviations and instrument

Fig. 1. Overview of colour scans and XRF maps of Vaccinites vesiculosus (VV, 1A), Torreites sanchezi milovanovici (TS, 1B) and Oscillophora figari (OF, 1C) sections. Images on the left show colour scans of the polished surface of the bivalves with locations of the lines of measurement in yellow. Central images show XRF maps of relative intensities of Fe (red), Si (green) and Ca (blue). Images on the right-hand side show XRF heatmaps of the relative intensity of Fe and Mn, with red colours indicating higher concentrations and blue colours indicating lower concentrations (see scale bar in 1A). Fig. 1B and C contain close-ups showing growth microstructure of the calcite in the outer shell layer and the hinge of the TS and OF shells, respectively. Background of the VV maps (1A) shows glass beads used to stabilize the shell during measurement. This was not necessary for the measurement of the heavier TS and OF shells (1B and 1C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Errors are reported in Table 1 and were calculated according to Winter et al. (2017). Data of all calibrated trace element and stable isotope measurements used in this study are provided in Supplementary data 2.

3.5. Stable isotopic analysis

Pristine calcite in cross sections of all three bivalve shells was sampled for stable isotope analysis using a microscope guided drill (Merchantek/Electro Scientific Industries Inc. (ESI), Portland (OR), USA, coupled to Leica GZ6, Leica Microsystems GmbH, Wetzlar, Germany) equipped with a 300 μm wide tungsten carbide drill bit. Samples for stable isotope analysis were taken at a resolution of 500 μm for TS (243 measurements) and 250 μm for VV and OF (328 and 92 measurements respectively). The spatial sampling resolution was decided upon based on the presence of annual growth bands and recognized annual cycles in trace element ratios measured by μXRF, and was chosen such that each interpreted year contained at least four samples. Samples for stable isotope analysis were taken along the same transects as XRF and LA-ICP-MS measurements (see Fig. 1). In the OF shell, all samples for stable isotope analysis were taken in the dense foliated calcite layers which are concentrated in the hinge of the left valve of the shell (Fig. 1). Sample aliquots with a typical weight of 30–50 μg were reacted with phosphoric acid (H3PO4·H2O) at 70 °C in a Nu Carb carbon preparation device coupled via dual inlet to a Nu Perspective isotope ratio mass spectrometer (Nu Instruments, UK) and the resulting CO2 was analyzed for stable carbon and oxygen isotope ratios. Analytical uncertainty was determined by repeated measurement (N = 194) of the in-house reference material MAR2 (Marbella marble, δ13C: 3.41 ± 0.10 ‰ VPDB; δ18O: 0.13 ± 0.20 ‰ VPDB; 1 standard deviation, SD) and found to be 0.05 ‰ and 0.09 ‰ for δ13C and δ18O values (1 SD), respectively. This MAR2 reference material was previously calibrated using the international NBS-19 stable isotope standard (Friedman et al., 1982). All stable isotope values are reported in permille relative to the Vienna Pee Dee Belemnite standard (‰VPDB).

3.6. Statistical analyses

Statistical analyses were performed to assess the possible presence of seasonal cyclicity in the records. To test for periodic behaviour in proxy records, periodograms were generated by R 3.1.2 software package (R Development Core Team, 2008) using three methods of spectral analysis in a frequency range of 10−5 and 10−3 μm−1: 1) A Lomb-Scargle Periodogram (LSP, see Lomb, 1976; Scargle, 1982) was calculated from untreated datasets with an oversampling factor of 100 and a confidence level of 90%. 2) A Multi Taper Method Spectral Analysis (MTM, see Thomson, 1982) was carried out on uniformly resampled and linearly detrended datasets.
using the R “astrochron” package by Meyers (2014). 3) A simple periodogram (SP, see Bloomfield, 2004) was calculated from uniformly resampled and linearly detrended datasets using R core functions. Confidence levels for detected frequencies were calculated via “red noise” estimation and harmonic F-test calculation (Thomson, 1982) using functions in the R “astrochron” package by Meyers (2012) and significant periodicities were extracted from the records by bandpass filtering (Hays et al., 1976) using the “bandpass” function in the R “astrochron” package (Meyers, 2012) on untreated datasets using a Cosine-tapered bandpass and a zero-padding factor of 5. The script used for these spectral analysis routines including the parameters that were used and the resultant data of filters, powerspectra and confidence levels is given in Supplementary data 6.

Table 1 Overview of measured average concentrations, measurement reproducibility (1 standard deviation, SD) and instrumental uncertainty (1 SD) based on XRF deconvolution for major and trace element measurements on the Vaccinites vesiculosus (VV) shell using the two XRF methods (conventional line scanning and point-by-point analysis) and LA-ICP-MS. For LA-ICP-MS, the coefficient of determination ($R^2$) of the calibration curve based on standard measurements is given.

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<th>Instrument error (ug/g)</th>
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</tbody>
</table>

4.2. Trace element concentrations

Fig. 3 provides an overview of the range in concentrations of major and trace elements measured in all three shells by LA-ICP-MS-calibrated XRF point-by-point line scans. Obtained concentrations of all measured elements fall within the same order of magnitude between different shells, with the exception of Fe. Even though an effort was made to avoid inclusions of Mn and Fe based on their location in Fig. 1 (see above), the VV record contains higher values for Fe, Mn and Si. Concentration ranges of other elements are mostly similar between shells.
Fig. 3. Average concentrations of all trace elements measured in the VV (purple), TS (blue) and OF (orange) shells using LA-ICP-MS calibrated point-by-point XRF measurements. Bars indicate ranges of concentrations measured in the shell calcite and black dots indicate the averages of these ranges. Values below 1 ppm are dashed because these are below the detection limit of the method (de Winter and Claeys, 2016). Elements of interest for this study are highlighted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The expression of these fluctuations in trace element records, especially those of Zn and S, is in general not sinusoidal but rather shows a punctuated character with rapid shifts in concentration and longer episodes of relative stability. Trace element records often co-vary (most notably Mg/Ca and Zn/Ca) or show anti-correlations (e.g. Zn/Ca and S/Ca). The Sr/Ca record of OF is characterized by a pronounced ontogenetic increase and shows a large drop at the end of the record. Fluctuations in the Sr record of OF are smaller than in the rudistid shells. Another ontogenetic trend in OF is observed in the S/Ca record, which shows much less variation in the initial ±6 mm of the record followed by an increase in fluctuation for the remainder of the record. A similar, though less pronounced, intensification of the trace element fluctuations is observed in the records of Mg/Ca and Zn/Ca.

4.3. Stable isotope ratios

Figs. 4–6 show that stable carbon and oxygen isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) fluctuate periodically around a stable background in all bivalve shells. An exception to this is the last part of the $\delta^{13}C$ record in OF (Fig. 6) where a decreasing trend is observed. While $\delta^{13}C$ values are similar between shells (±0.5‰ to ±2.5‰ with a mean value of ±1.5‰ in VV and TS and ±2‰ in OF), large inter-shell differences are observed in $\delta^{18}O$ values. Oxygen isotope ratios are highest in OF (roughly between −4‰ and −3‰ with a mean value around −3.5‰) and significantly lower in TS (−6‰ to −5‰, mean value around −5‰) with intermediate values for VV (between −5‰ and −3‰ with a mean value of −4‰). Fluctuations in stable isotope values also vary between shells. Measured $\delta^{13}C$ values fluctuate with an amplitude of approximately 1‰ in VV and OF, while fluctuations in TS are about 1.5‰. Oxygen isotope values fluctuate from 1‰ to 1.5‰ in TS and OF, but are closer to 2‰ in VV. Stable isotope fluctuations seem to follow the same periodicity as the trace element records (10–20 mm in rudistid shells, 2–3 mm in OF) and relationships between these records are described in paragraph 4.5. Fluctuations in the stable isotope records are more regular and have a more constant amplitude compared to trace element records.

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4.4. Seasonal cyclicity

Relatively scaled powerspectra of three methods of spectral analysis (MTM, Lomb-Scargle and simple periodogram) are plotted in Figs. 7, 8 and 9. Confidence levels for detected frequencies show that frequencies with high spectral power according to all spectral analysis methods (black rectangle in Figs. 7–9) are generally associated with high (>80%) estimated confidence levels. These are the frequency ranges that are filtered from the records using bandpass filtering. Resulting bandpass filters in the frequency domain of seasonality pick up fluctuations in the records that were identified in Figs. 4–6. Figs. 7–9 show that the period best describing the data is ±8 mm long in VV, ±20 mm in TS and ±3 mm in OF. Results of spectral analysis show that all records show periodicity in the seasonal range, but cyclicity is, in general, best represented in Sr/Ca, Mg/Ca and stable isotope records, where bandpass filters most successfully pick up the cyclicity in the records. Results from the three different methods of spectral analysis are not similar in all cases but agree, in general, in the frequency domain where the seasonal cycle is found. Bandpass filters of the proxy records reflect the periodic variation in stable isotope records, but fail to explain the majority of the variation in trace element records, which have a less sinusoidal character. Furthermore, interpretation of growth seasons in Figs. 7–9 show that the amount of calcite deposition in the shells is not equal between seasons, resulting in larger parts of the TS shell being deposited under high-δ18O conditions and slightly larger parts of the VV shell deposited under low-δ18O conditions. There is no observable difference in the length of seasons in the OF shell. Interpretation of seasonal cyclicity in the three shells yields an age of 9 years for OF, 6 years for TS and 8–9 years for VV. Age of VV is uncertain due to the poorly preserved lower ±20 mm which precluded isotope analysis, forcing the growth period to be estimated to 2–3 years for this part of the shell (see Fig. 7).

4.5. Phase relationships

As indicated in Figs. 4–9, periodic fluctuations of stable isotope and trace element records can be traced between different proxy records.
An overview of visually observed and statistically significant correlations between proxy records of the bivalves is given in Table 2. Figs. 7–9 show that many proxies only show clear phase relationships in parts of the record and correlations between total shell records are generally low (see Table 2), as proxies shift in and out of phase. One example of this is found in the VV shell, where periodicity in Zn/Ca, δ^{13}C and Sr/Ca is most pronounced in the central part of the record (30–70 mm; Fig. 7). Another case is in the OF record where the last 2 mm are influenced by a strong trend in δ^{13}C and Sr/Ca, whereas the part of the record that immediately precedes this drop (i.e., 14–22 mm) shows clear periodic variation. A close inspection of the phase relationships between proxies (Figs 7–9) demonstrates that periodicities in δ^{18}O records are
Fig. 6. Oscillopha figari (OF) shell. Overview of stable isotope (‰ VPDB) and elemental abundance records (wt% and μg/g) and relative abundance records (mmol/mol, 11 point running average). Dashed lines marked "I", "II" and "III" correspond to marks on the sampling lines in Fig. 1C.
generally in phase with Mg/Ca. However, this relationship is only statistically significant in OF (Table 2). On the other hand, the positive relationships between Mg/Ca and S/Ca that are visually observed in all shells do have a statistical basis. Positive correlation of Sr/Ca with S/Ca is both visually observed and statistically significant in the rudistid shells, but not in the oyster shell. Another difference between the rudistids and the oyster is a negative correlation of \( \delta^{13}C \) with S/Ca and Sr/Ca in the rudistid shells, which in the OF shell is positive. The latter is presumably caused in large by the sharp drop in Sr/Ca and \( \delta^{13}C \) near the end of the record. Sr/Ca ratios seem to be in antiphase with Mg/Ca ratios in most shell records, but this correlation is only weakly significant in TS and OF. In the TS shell, contrary to the other two shells, both stable isotope records are strongly correlated. The relationship of Zn/Ca with other records is complicated, as not many correlations have statistically significant \( p \)-values (\( p < 0.05 \)). Pearson’s coefficients (\( r \)) indicate that Zn/Ca ratios often exhibit weak positive correlations with Mg/Ca and negative correlations with Sr/Ca. However, these relationships are not always supported by visual inspection, mostly because the phase relationship of these records tends to change across the record (see Figs. 4–9). Interestingly, visual inspection suggests a negative relationship between Zn/Ca and S/Ca, especially in the parts of VV and OF that show well-expressed periodicity. However, this relationship is only significant in the bulk statistics of the TS record. This again indicates that phase relationships between these proxies are complex, change throughout the records and cannot easily be summarized by a statistical value.

5. Discussion

5.1. Shell preservation

5.1.1. Spatial distribution

In the case of fossil bivalve taxa, a map of Fe, Si and calcium (Ca) concentrations highlights zones in the sample, where the calcite underwent diagenetic overprinting. As such, Fig. 1 provides an initial survey of the state of preservation of the bivalve shells. In addition, a heatmap of the combined intensities of Fe and Mn (elements both associated with diagenesis, see Al-Aasm and Veizer, 1986a, 1986b; Steuber, 1999), highlights those spots where diagenetic carbonates, (re-)crystallized post-mortem, are most common. XRF maps indicate that the best-preserved calcite is found in the outer shell layers of rudistid shells. As discussed by de Winter and Claeys (2016), this layer is punctuated by zones of diagenetic material that should be avoided during sampling. In the VV shell, the left part of the shell cross-section contains a higher amount of these spots affected by diagenesis than the right part and spots are present in greater concentration in the lower part of the shell (as oriented in Fig. 1). The concentrations of Ca, Si, Fe and Mn in the sampled parts of shells (Figs. 3–6) indicate that diagenesis, although present to some extent in all shells, is more localized and therefore easier to avoid during sampling of TS and OF. This explains why the VV record contains more intervals with increased concentrations of Si, Fe and
Mn (see also Fig. 3). Another difference between the VV shell and TS and OF is the relative concentrations of Si, Fe and Mn in altered parts of the shell (Compare Fig. 4 with Figs 5 and 6). This difference may be explained by two different types of diagenesis that affected the fossils.

5.1.2. Boring

The first is represented by the highly Fe and Mn enriched spots in VV, which can be explained by boring into the shell by predators (most likely sponges; Sanders and Baron-Szabo, 1997). Bore holes are later filled in by detrital material rich in Fe, Mn and Si (see de Winter and Claeys, 2016). These bore holes explain the drops (by several percent) in concentration of Ca observed in Fig. 4, where calcite is replaced by (Fe-, Mn- and Si-rich) inclusions of detrital material that filled up the bore holes. This boring into the bivalve shells most likely took place during the life time of the bivalve or early post-mortem and has most strongly affected the VV shell. The relatively thin outer shell layers of VV probably made it more susceptible to boring predation than the significantly more thick-shelled TS and OF (Sanders and Baron-Szabo, 1997), explaining more frequent bore holes in the VV shell and higher local Fe and Mn concentrations (see also Fig. 3).

5.1.3. Silification

The second type of diagenesis is silification, which is evident from the corona of Si-rich material that is present on the outside and in the cavity of the TS shell and to a lesser extent on the OF shell (Fig. 1). This silification took place after burial of the fossils, is strongly controlled by the local concentration of Si in the pore fluid (Maliva and Siever, 1988) and has left large (mm- to cm-scale) euhedral quartz crystals in the porous cavity of the TS shell (Fig. 1). The fact that VV was relatively unaffected by silification seems counter-intuitive, as VV and TS shells are stratigraphically more closely associated with each other than with OF, which was found slightly lower in the stratigraphy. However, since the V1-member of the Samhan Formation, from which VV and TS originate, is laterally continuous for 200 m (Schumann, 1995), it cannot be excluded that local differences in Si content of the pore fluid in this member were large enough to cause the observed difference in the extent of silification between the shells. While traces of silification are also visible in OF, it is hypothesized that the calcite shell of this bivalve was likely more resistant to replacement by quartz than the partly aragonitic TS shell (see Schubert et al., 1997), preventing the extensive (cm-deep) invasion of Si-rich pore fluid observed in the TS shell from affecting OF. The last 2 mm of the Sr/Ca record in the OF shell (Fig. 6) were probably affected by diagenesis as drops in Sr concentrations are much more severe than those observed in pristine modern
bivalves. The sharp decrease in Sr/Ca is matched by a decrease in δ¹³C, potentially indicating incorporation of ¹²C–enriched carbonate during diagenesis. The statistically significant positive correlation between Sr/Ca and δ¹³C (Table 2) is strongly controlled by this diagenetic drop at the end of the record.

5.1.4. Data filtering

In Fig. 4(VV), parts of the trace element record that show peaks in the concentrations of these elements were removed from records of Mg/Ca, Zn/Ca, Sr/Ca and S/Ca to avoid interpretation of parts of the record that were affected by diagenesis. This was not necessary in the records of TS and OF as the Mn and Fe concentration in these shells mostly remained well below 200 μg/g, which has been proposed as a threshold for pristine shell material (Brand and Veizer, 1980; Al-Aasm and Veizer, 1986a, 1986b; Steuber, 1999). However, as is evidenced by the presence of silicification in these shells, thresholds of Mn, Fe and Sr concentrations as indicators for pristine carbonate preservation are highly simplistic and can only be used to trace calcite recrystallization from reducing pore fluids (Aller, 1980; Brand and Veizer, 1980). Furthermore, fossilization of the bivalves has probably limited the preservation of organic matter in the shells, as indicated by the lower S concentrations found in the present study (100–200 μg/g) compared to higher (~500 μg/g) S concentrations found in modern bivalve shells (Lorens and Bender, 1980; Dalbeck et al., 2006). Microscope-guided drilling for stable isotope samples guided by XRF mapping allowed bore holes and silicified shell parts to be avoided, and the resulting filtered trace element and stable isotope records (Fig. 4–6) are indicative for well-preserved bivalve calcite.

5.2. Multi-proxy records

5.2.1. Comparison with modern shells

Element-to-Ca ratios of Mg, Zn and Sr of fossil shells in this study are in agreement with ratios found in modern bivalves (e.g. Carriker et al., 1980; Al-Aasm and Veizer, 1986a, 1986b; Steuber, 1999; Vander Putten et al., 2000; Holmden and Hudson, 2003). Mg/Ca and Sr/Ca in OF are similar to those found in modern oyster species (Carriker et al., 1980; Ullmann et al., 2013; Mouchi et al., 2013), though with lower Mg/Ca than found in the estuarine oyster Crassostrea virginica (Surge and Lohmann, 2008). Concentrations of Zn, Mn and Fe are also in close agreement with those found in modern oyster shells (Carriker et al., 1980; Ullmann et al., 2013), and S concentrations are similar to those found in foliated calcite structures of Crassostrea gigas by Dauphin et al. (2013), though slightly lower than those found in other marine bivalves such as Mytilus edulis (Lorens and Bender, 1980; Dalbeck et al., 2006). Trace element concentrations are generally higher in VV and TS, and are in closer agreement with those in calcite shells of modern marine bivalves (Vander Putten et al., 2000; Gillikin et al., 2005; Freitas et al., 2009), with the exception of S, which is lower than found in calcite of marine bivalves (Lorens and Bender, 1980; Dalbeck et al.,...
Table 2
Overview matrix of correlations between proxy records in the three bivalves. Upper matrices show correlations made on the basis of visual inspection. Red fills indicate antiphase correlations while green fills signify in-phase correlations. Matrices in the second row show p-values of correlations between these records. Green fills indicate statistically significant relationships (p < 0.05), while red fills indicate no significant relationship. The bottom row of matrices show Pearson’s coefficients (r) of correlations between records. Green fills indicate positive correlations between records (r > 0) and red and orange fills indicate negative correlations (r < 0). White fills indicate that there is no significant correlation between the records.

<table>
<thead>
<tr>
<th>Vacciniteas vesiculosus (VV)</th>
<th>Torreites sanchezi milovanovici (TS)</th>
<th>Oscillopha figari (OF)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visual</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
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<tr>
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<td>Mg/Ca</td>
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the proxy records. Interpretation of seasonal cycles in these trend. This illustrates that it remains important to visually inspect and in OF is weak, its correlation is controlled by the stronger ontogenetic indicators that shell growth varies seasonally. The length of the deposited in a year is variable, generally becoming shorter with shell age, an observation that is in agreement with observations in modern bivalves (Surge and Lohmann, 2008).

5.2.2. Phase relationships

The complexity of phase relationships in this multi-proxy study is illustrated by the lack of significant, strong (p < 0.05; r > 0.60) correlations between proxy records (Table 2). This lack of linear relationships is caused by different parameters acting on the proxies at different times and because the expression of seasonality in the shells is not always equally strong (Figs. 7–9). A close examination of Figs. 7–9 shows that this is indeed the case, as filtered sinusoidal trends only explain a part of the variation. The amplitude of seasonality observed in proxy records is also highly variable along the length of the shell records, and on many occasions this seasonality (especially in trace element records) is overprinted by short-lived variations, obscuring phase relationships between proxies. Finally, the amount of shell material deposited in a year is variable generally becoming shorter with shell age, an observation that is in agreement with observations in modern bivalves (e.g. Richardson et al., 2004; Schöne et al., 2005, 2011).

5.3. Bivalve physiology

5.3.1. Ontogenetic trends

Despite the fact that bivalves in this study were relatively short-lived, some ontogenetic trends are observed in the record. The gradually decreasing trend in Sr/Ca in the OF shell is similar to ontogenetic trends in Sr/Ca found in modern oysters (Ullmann et al., 2013), suggesting an effect of growth rate on the incorporation of Sr into OF. Discarding the final drop in δ13C, no significant ontogenetic trend in δ13C values is observed in OF. On a seasonal scale, lows in Sr/Ca coincide with high δ13C (Figs. 7 and 9), indicating that differences in metabolic rate may have controlled Sr/Ca ratios, as observed in culture studies on modern bivalves (Lorrain et al., 2005; Freitas et al., 2006). Since Sr/Ca seasonality in OF is weak, its correlation is controlled by the stronger ontogenetic trend. This illustrates that it remains important to visually inspect and compare seasonal records to complement bulk statistical analysis of the proxy records. Interpretation of seasonal cycles in these figures further indicates that shell growth varies seasonally. The length of the interpreted warm-low-salinity season (lower δ18O) and cold/higher-salinity season (higher δ18O) are not equal, showing differential growth rates between the seasons or even complete growth halts during extreme conditions (Wanamaker et al., 2011). The difference in the amount of shell material deposited during different seasons indicates either that the records of TS was more strongly affected by extreme conditions in the warm-low-salinity season than the other shells, or that its growth conditions were more extreme in terms of high temperatures and/or low salinities than those of VV and OF. The former hypothesis might indicate that TS was more resistant to extreme conditions, but the latter hypothesis cannot be excluded because the length of these short-lived bivalve records means that the probability of these organisms living at the same moment in time is small. Thickness of annual deposits in the shells decreases with age, suggesting ontogenetically decreasing growth rates, in agreement with other studies of rudistids (Steuber, 1995, 1996) and oysters (Ullmann et al., 2010). Periodic fluctuations in the stable isotope records of the OF shell are paced to the growth increments observed in Fig. 1, showing that these growth increments are likely annual in Osilinophia fragilis, contrary to those observed in modern Crassostrea gigas (Ullmann et al., 2013).

5.3.2. Physiological control on trace element incorporation

The correlation between Mg/Ca and Sr/Ca found by Lorenz and Bender (1980) is also observed in the bivalve records in the present study (Table 2), and is especially clearly visible in the OF record. S is present in the organic matrices of bivalve calcite in the form of ester sulphates and comprises approximately 10% of the organic matter in the shell, meaning that S/Ca ratios of up to 10 mmol/mol can be explained by the occurrence of S in organic matter in the shell (Crenshaw, 1972; Lorenz and Bender, 1980). However, more recent studies have concluded that a large fraction of S in bivalve shells is present in inorganic form (Yoshimura et al., 2014). Annual fluctuations in S contents in extant aragonitic and calcitic bivalves have been linked to growth rate and concentrations in the calcifying fluid, with lower S concentrations during more rapid growth (Yoshimura et al., 2013, 2014). These fluctuations become progressively more pronounced with age in the OF shell, showing that perhaps these short episodes of rapid growth become more common with age in the oyster shell. This increase in fluctuation of the S/Ca record is mirrored in the Mg/Ca record. The covariances of S/Ca with Mg/Ca can be explained by local increases in the amount of organic matrix in the carbonate structure of the shell. It has been shown that bivalves secrete additional sulphate-bearing organic matter under the influence of crystal growth-inhibiting concentrations of Mg in order to seed calcite growth by locally increasing Ca concentrations, causing local enrichments of Mg and S in the shell (Lorenz and Bender, 1980). This physiological control on Mg incorporation might mean that Mg concentrations, whenever correlated to S concentrations, are associated with the organic matrix of the shell rather than the calcite, implying that caution must be exercised when interpreting Mg/Ca ratios in terms of palaeotemperature (Rosenberg and Hughes, 1991; England et al., 2007). Alternatively, if part of the Mg in the shells is bound to the organic matrix rather than the calcite lattice, increases in S/Ca and Mg/Ca ratios may indicate calcite deposited during times of stress, when more organic matter is secreted (Lorenz and Bender, 1980; Rosenberg et al., 1989, 2001). Such periods of increased stress may also be controlled by growth rate or environmental stress, as episodes of rapid growth or outside stress may rupture the periostracal rim resulting in increased organic matrix production and allowing sulphate-rich sea water to enter the extra-pallial fluid (Carriker et al., 1980; Skelton et al., 1990; Rosenberg et al., 2001). This hypothesis is consistent with the occurrence of storm events in the turbulent environmental setting of the bivalves, which could have caused disruption of the bivalve’s life environment on a regular basis.

5.4. Sea water chemistry

5.4.1. Zn/Ca ratios and palaeoproductivity

Parts of all shell records show periodic spikes in Zn/Ca, indicating that there were seasonal changes in productivity in the environment. The punctuated character of the Zn/Ca record, with sharp increases rather than more sinusoidal fluctuation, can be explained by episodes of release of Zn into the environment after productivity blooms. The fact that Zn concentrations are lower during the remainder of the record is ascribed to its role as a micronutrient. It is continuously taken up by primary producers and bound to organic ligands without being replenished fast enough unless during times when respiration increases dramatically, i.e. after productivity blooms (Bruland, 1989; Calvert and Pedersen, 1993; Vance et al., 2017). If Zn concentrations in the shell increase after episodes of increased productivity, as shown in modern environment studies (Calvert and Pedersen, 1993; Jackson et al., 1993; Guo et al., 2002), Zn/Ca fluctuations record blooms of
palaeoproducitivity in the shallow marine environment of the shells.
This also explains why often multiple peaks in Zn/Ca ratios per year are observed, especially in the OF and TS shell records, which display double peaks in Zn/Ca paced to seasonal variation in δ¹⁸O records. These increases in palaeoproducitivity likely strongly affected the local surface ocean chemistry, especially in the progressively more confined late Cretaceous Tethys Ocean (Dercourt et al., 1986; Almogi-Labin et al., 1993; Steuber and Löser, 2000; Jarvis et al., 2002; Fleurance et al., 2013). Indeed, Zn/Ca peaks in VV and OF seem to be paced to intervals with higher δ¹³C values, which may reflect increased δ¹³C values in the water indicative of increased productivity (Grossman, 1984; Jarvis et al., 2002; Takesue and van Geen, 2004) or decreased metabolic activity (Klein et al., 1996b; Owen et al., 2002; Gillikin et al., 2006). The latter may be explained by changes in the redox state of the shallow ocean as a consequence of the reduction of organic matter after a productivity bloom. It has been proposed that this relationship between metabolic rate and δ¹⁳C values is especially strong during periods of relatively low respiration and low ambient CO₂/O₂ ratios, as is the case in well-oxygenated waters (McConnaughey et al., 1997; Gillikin et al., 2006). The observed anti-phase relationship between Zn/Ca and Sr/Ca in VV and OF supports this hypothesis of reduced metabolic rate in the shells during periods corresponding to peaks in Zn/Ca ratios. Such seasonal changes in redox conditions in the surface ocean are common in present-day low-latitude settings (Morrison et al., 1998). Under more oxygen-poor conditions, the mobility and bioavailability of redox sensitive elements in the water column increases significantly (Zwolsman et al., 1997; Gerringa et al., 2001). Seasonal cycles in Zn/Ca and Sr/Ca are especially evident in the OF shell, showing that changes in Zn availability are readily recorded in oyster shells, as shown by Carriker et al. (1980) and Guo et al. (2002). Large S/Ca shifts show that seasonal changes in the organic matter fraction incorporated in the shell of OF are more pronounced than in the rudistid shells.

5.4.2. S/Ca ratios and sea water chemistry

In parts of VV and OF where Zn/Ca shows well-expressed periodicity, the record is in antiphase with S/Ca ratios, which might be interpreted as evidence for an environmental control mechanism on S concentrations in the shells (Figs. 4, 6, 7, 9). One hypothesis might be that more reducing conditions allow S to be precipitated in sulphides while more oxidizing conditions can remobilize S from the sediment, increasing S concentrations (Ku et al., 1999; Kah et al., 2004). However, since the sulphate concentration in ocean water is several orders of magnitude higher than the concentrations of S found in the present study, it is unlikely that changes in redox conditions in this shallow, open-marine system could have brought about conditions where this source would be limited (Chester, 2009). Evidence of a highly turbulent environment in which these bivalves lived renders severe redox stratification unlikely (Schumann, 1995). Furthermore, a redox shift that allows the precipitation of sulphides would also reduce the bioavailability of Zn by means of precipitation of Zn sulphides, and this is not consistent with the observed antiphase relationship between Zn/Ca and S/Ca (Calvert and Pedersen, 1993; Guo et al., 1997). Furthermore, the precipitation of ZnS requires more reducing conditions than may be expected in the shallow marine, turbulent environment of the bivalves in this study (Guo et al., 1997; Schumann, 1995). Instead, it is more likely that a change in growth and metabolic rate caused by increases in productivity, when Zn concentrations are highest, resulted in an increase in S/Ca ratios.

5.5. Palaeotemperature seasonality

5.5.1. Stable oxygen isotope ratios

In several studies, relationships have been found between chemical proxies (Mg/Ca, Sr/Ca and δ¹⁸O) in biogenic carbonates and the calcification temperature of these carbonates (e.g. Epstein et al., 1953; Craig, 1965; O’Neil et al., 1969; Tarutani et al., 1969; Hays and Grossman, 1991; Takesue and van Geen, 2004; Wanamaker et al., 2008). Since other factors (e.g., salinity, growth rate and water chemistry) also appear to control these proxies in molluscan shells, different proxies need to be compared to isolate the effect of temperature. Temperature transfer functions were either obtained through controlled inorganic calcium carbonate precipitation (e.g., Epstein et al., 1953; O’Neil et al., 1969; Kim and O’Neil, 1997) or from culture experiments and comparison of proxy data with historical temperature records in bivalves as well as in other calcifying organisms (e.g., de Villiers et al., 1995; Freitas et al., 2005; Takesue and van Geen, 2004; Surge and Lohmann, 2008; Wanamaker et al., 2008). For palaeotemperature reconstructions based on δ¹⁸O values, a value of ~1‰ is assumed for Late Cretaceous sea water (Savin and Yeh, 1981; Steuber, 1995). Temperature estimates of minima and maxima of the seasonal cycle based on stable oxygen isotope ratios from shell OF and VV yield temperatures of 25 °C and 32 °C respectively within a 1–2 °C range depending on the calibration used (e.g. Epstein et al., 1953; Craig, 1965; O’Neil et al., 1969; Tarutani et al., 1969; Hays and Grossman, 1991). Minimum seasonal temperatures based on δ¹⁸O values in the OF and VV shells are in agreement, while maximum seasonal temperatures recorded in VV (~34 °C) exceed those determined for OF (~30 °C). Temperatures for the TS shell using the same parameters turn out higher, ranging between 34 °C and 43 °C. Temperatures determined based on OF and VV are in agreement with temperatures found for Vaccinites cornucaucim, Vaccinites ultimus, Vaccinites oppelti, all of which are in the same family as VV, as well as to temperatures derived from other rudistid taxa from the Upper Cretaceous (Steuber, 1995, 1996, 1999). Temperatures found in the seasonal cycle of TS overlap with those documented for other rudistids, but are still exceptionally high (Steuber et al., 2005), suggesting that δ¹⁸O values in this rudistid may have been controlled by other factors besides temperature. Seasonal changes in salinity could account for a change in stable oxygen isotope ratios (Klein et al., 1996a). The distal, open marine environmental setting of these shells, however, precludes a strong riverine component to explain large changes in salinity. Because of the relatively high δ¹⁸O values of rainfall in tropical climates, changes in salinity in the order of 10 g/kg would be required to account for a 1‰ difference in δ¹⁸O in the TS shell (Ravelo and Hillaire-Marcel, 2007). However, it cannot be excluded that seasonal salinity changes did influence the stable isotope records of the shells differently, primarily because it is not certain exactly how much time existed between the lifetimes of the bivalves. Furthermore, as speculated above, it is likely that extreme periods of the seasonal cycle were not recorded in one species while they were recorded in another. The preferential exclusion of part of the seasonal cycle from the TS shell (in this case the ¹⁸O-depleted warm/low-salinity part of the cycle) would explain the difference in seasonality between the δ¹⁸O record of this shell and that of the closely associated VV, but does not explain the 1‰ difference in mean value between the records. Differences in seasonality between the rudistid shells and the OF are probably explained by actual changes in climate between their lifetimes, as a significant amount of time may separate their formation. The fact that δ¹⁸O records in modern oysters seem to faithfully record water temperatures (if δ¹⁸O of seawater is well-constrained; see Surge and Lohmann, 2008; Ullmann et al., 2010) puts slightly more confidence in the temperatures reconstructed from the VV shell. Although isotope ratios from Torreites shells from the same region have been interpreted to fractionate in disequilibrium with respect to ambient sea water (Steuber, 1999), the data in this study cannot confirm or disprove this statement. However, since disequilibrium fractionation of isotopes in marine calcitic bivalve shells is rare, it cannot be excluded that TS precipitated its calcite under significantly lower-salinity (~10 g/kg) or higher temperature (~8 °C) with respect to the other two bivalves. Furthermore, it must be stated that the use of one specimen for each species in this study precludes the discussion of specimen–specific effects and therefore adds a certain uncertainty to the reconstructions. This uncertainty is somewhat alleviated by the agreement of the temperature reconstructions in this study with
other bivalve-based (Steuber, 1995, 1996, 1999) and other reconstructions (e.g. Huber et al., 1995; Brady et al., 1998; Alsenz et al., 2013).

5.5.2. Mg/Ca ratios

Even though Mg/Ca ratios have been proposed as a proxy for calcification temperature in a range of calcifying organisms (e.g. Klein et al., 1996a; Vander Putten et al., 2000; Elderfield and Ganssen, 2000; Takesue and van Geen, 2004; Freitas et al., 2005; Wanamaker et al., 2008; Surge and Lohmann, 2008; Cleroux et al., 2008; Mouchi et al., 2013), empirically determined dependencies of Mg concentrations on sea water temperature as well as mean concentrations of Mg in these biogenic calcites differ strongly, causing widely different transfer functions between these studies, as illustrated in Fig. 10. The fact that Mg/Ca ratios in the OF shell are systematically lower than those from the rudistid shells shows that applying the same transfer function for these shells would lead to different palaeotemperature reconstructions. Using oyster-based calibrations to calculate temperatures from Mg/Ca ratios in the OF shell yields unrealistically cold mean annual temperature estimates of 2.9 °C (Surge and Lohmann, 2008) and 8.8 °C (Mouchi et al., 2013) with a seasonal temperature variability of ±2.5 °C and ±6 °C, respectively. A probable reason for these underestimations of temperature with respect to the temperature reconstructions based on δ18O is that the Mg/Ca ratio of ocean water in the Late Cretaceous (1–2 mol/mol; Stanley and Hardie, 1998; Coggon et al., 2010) was lower than it is today (5 mol/mol; Stanley and Hardie, 1998). While the incorporation of trace elements into bivalve calcite is not yet fully understood, it seems likely that concentrations in ambient sea water exert a dominant control on the concentrations in the shell. Taking this estimated decrease in sea water Mg/Ca ratios by a factor of 3.3 with respect to present-day values into account and recalculating temperatures based on Mg/Ca ratios in the OF shell yields mean annual palaeotemperature estimates of 9 °C with a temperature seasonality of 10 °C using the Surge and Lohmann (2008) calibration and 25 °C with a temperature seasonality of 14 °C for the Mouchi et al. (2013) calibration. These mean annual temperature estimates, especially those based on Surge and Lohmann (2008), are still much lower than those based on δ18O values, and temperature seasonalities of 10 °C or more seem unrealistic for tropical ocean water. When this correction for different sea water Mg/Ca ratios is applied on the Mg/Ca records of VV and TS, reconstructed seasonalities based on bivalve calibrations (Klein et al., 1996a; Vander Putten et al., 2000; Takesue and van Geen, 2004; Wanamaker et al., 2008) are unrealistically high as well (>10 °C), while mean annual temperatures vary widely depending on the calibration that is used (see Fig. 10). It is possible that the temperature dependence of the incorporation of Mg into bivalve calcite was different in low-Mg/Ca oceans or in the much hotter climate of the Late Cretaceous, or that a part of the Mg in bivalve shells is associated with the organic matrix in the shell and controlled by physiological processes rather than ambient temperature (Freitas et al., 2009; see Discussion in Section 5.3). In any case, the present exercise shows that palaeotemperature reconstructions based on the Mg/Ca proxy need to be treated with care until the mechanisms controlling the incorporation of Mg into the shell is better understood.

5.5.3. Sr/Ca ratios

Palaeotemperature estimates based on Sr/Ca ratios in bivalve calcite also need to be treated with care, since previous studies have shown that changes in Sr/Ca ratios correlate with changes in growth and metabolic rate (Klein et al., 1996b; Lorrain et al., 2005; Freitas et al., 2006) and salinity (Klein et al., 1996b; Wanamaker et al., 2008). Nevertheless, some authors have proposed relationships between Sr/Ca ratios and sea water temperature (Takesue and van Geen, 2004; Wanamaker et al., 2008). Calibrations from both these studies show a positive correlation of Sr/Ca with temperature, while a negative relationship would be expected based on experimental data and thermodynamic considerations (Rimstidt et al., 1998). Applying these calibrations on this study’s Sr/Ca records leads to severely inconsistent estimates and consistent underestimation of sea water temperatures with respect to temperatures based on δ18O values (−13 °C to 30 °C mean annual temperatures according to the calibration of Wanamaker et al., 2008 assuming 32 g/kg salinity and 5 °C to 8 °C mean annual temperatures according to the Takesue and van Geen, 2004 intertidal aragonite shell calibration). Furthermore, the Wanamaker et al. (2008) calibrations yield unrealistically large seasonal temperature ranges. Correction for lower Sr/Ca ratios in Late Cretaceous ocean water or use of a low salinity model from Wanamaker et al. (2008) yields impossibly high temperature estimates (>100 °C). Using the calibration from Takesue and van Geen (2004) with sea water-corrected Sr/Ca values yields temperatures closer to temperatures based on δ18O, though much lower (mean annual temperatures range from 12 °C for OF to 14 °C for TS with temperature seasonalities of 1.5 °C for OF and 10 °C for the rudistids). Nevertheless, reconstructions based on Sr/Ca ratios vary significantly between specimens and are much lower than would be expected of this locality. These results illustrate the complexity of Sr/Ca ratios in bivalves and shows that they are not necessarily temperature dependent (e.g. Lorens and Bender, 1980; Klein et al., 1996b; Steuber, 1999). It is possible that the Sr/Ca is in part controlled by temperature, while much of the larger variation in Sr/Ca is caused by other processes such as the above-mentioned growth rate and salinity effects.

6. Conclusions

The study of three co-occurring extinct bivalve taxa sheds light on differences in the response of these bivalves to seasonally changing environmental conditions. μXRF line scanning on polished shell surfaces provides a method for simultaneous acquisition of multiple trace element records, including S, along the growth axis of the shells with a strong control on possible effects by diagenesis. A combination of these trace element records with stable carbon and oxygen isotope measurements illustrates the complex interplay of environmental and
physiological processes that control the incorporation of trace elements and stable isotopes into bivalve shells in this low-latitude setting in the late Campanian south eastern Tethys Ocean on the seasonal scale. Our results indicate that δ18O-based temperature reconstructions yield a seasonal temperature cycle of ±8 °C around a mean annual sea surface temperature of ±28 °C, which is comparable to the present-day situation in the oceans near Oman and in agreement with other reconstructions. Temperature reconstructions based on Mg/Ca or Sr/Ca ratios remain problematic in fossil bivalves, because of the vast differences in the transfer functions found for modern bivalves and the uncertainty in long-term fluctuations of trace element concentrations in the oceans. Correction of Mg/Ca ratios for reconstructed differences in sea water Mg/Ca ratios between the present and the Late Campanian yields temperatures that are close to those reconstructed via stable oxygen isotopes, but such reconstructions exhibit much larger seasonal differences, indicating that Mg/Ca ratios in these bivalves are controlled by more than just sea water temperature. The existence of physiological effects on Mg incorporation into these bivalve shells is evidenced by covarying Mg/Ca and Sr/Ca ratios. Palaeoecotemperature reconstructions using Sr/Ca ratios are different from those based on stable oxygen isotope ratios, leading to the conclusion that temperature dependence of this proxy is weak at best and that other factors such as growth rate, metabolism and salinity probably have strong effects on the Sr/Ca ratios. Zn records show that these Late Campanian shallow seas were characterized by productivity blooms, which control Zn concentrations in the water column analogous to present-day shallow-marine systems. After these blooms, enhanced respiration remobilized Zn in the surface ocean and decreased growth rates of the bivalves, leading to the decreased incorporation of S in their shells. The oyster species Ostrea edulis from the Oertijdmuseum De Groene Poort in Boxtel, the Netherlands and the Natuurhistorisch Museum Maastricht. NJW is financed by a personal PhD fellowship from IWT Flemens (IWT700). SG is a Postdoctoral Fellow of the Research Foundation Flanders (FWO, OZR199480F0). PC thanks the Hercules Foundation Flanders for an upgrade of the stable isotope laboratory (grant HERC0) and the acquisition of XRF instrumentation (grant HERC1309). The authors acknowledge financial and logistic support from the Flemish Research Foundation (FWO, project G017217N) and Teledyne CETAC Technologies (Omaha, NE, USA) as well as support from VUB Strategic Research (BAS48). SVM is a Ph.D. Fellow of the FWO. We thank Lieven Bekaert and Quintin Dierckens for their help with the sample preparation.

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