ASSESSING THE SUITABILITY OF NATIVE MOLLUSK SHELLS FOR SCLEROCHRONOCHEMISTRY STUDIES: AN ANALYSIS OF EIGHT PERSIAN GULF SPECIES

MELYIK CSIGA- ÉS KAGYLÓHÉJAK ALKALMASAK SZKLEROKRONOKÉMIAI VIZSGÁLATOKRA? – ÉRTÉKELÉS NYOLC, PERZSA-ÖBÖLBEN HONOS FAJ VIZSGÁLATA ALAPJÁN[•]

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Abstract

*This study aimed to identify suitable mollusk shells for use in sclerochemistry and sclerochronochemistry studies which, applied on shells in archaeological record, can help researchers reconstruct past climatic conditions. The shell features of eight commonly occurring native mollusk species in the Persian Gulf were examined. Four sea snails (*Bufonaria echinata *(Link, 1807),* Tibia fusus *(Linnaeus, 1758),* Architectonica perspectiva *(Linnaeus, 1758),* Cypraea turdus winckworthi *(F. A. Schilder & M. Schilder, 1938)) and four bivalves (*Vepricardium asiaticum *(Bruguière, 1789),* Callista umbonella *(Lamarck, 1818),* Saccostrea cucullata *(Born, 1778),* Anodonta cygnea *(Linnaeus, 1758)) were collected from their natural habitat in 2020, for analysis. The study used micro Raman spectroscopy to identify shells with an aragonite outer layer, a requirement for sclerochemistry research. Additionally, the study utilized sclerochronological methods to determine whether the shell samples could be appropriately studied for sclerochronochemistry research. Raman spectroscopy results indicated the presence of aragonite in all species, excluding* Saccostrea cucullata*. Pigments were also present on the surface of all shells except those of* Callista umbonella*. Cross-sectional analysis indicated that the shells of* Callista umbonella *exhibit clear daily, monthly, and annual growth lines with a thickness of 1650 μm in the outer layer, making them excellent candidates for sclerochemistry and sclerochronochemistry studies.*

Kivonat

*A tanulmány célja, hogy megfelelő puhatestű maradványokat azonosítsunk szklerokémiai és szklerokronokémiai vizsgálatokra, amelyek a régészeti leletekként előkerülő csigákból vagy kagylókból a klíma és az egykori környezet rekonstruálására alkalmasak. Nyolc, a Perzsa-öbölben gyakran előforduló, őshonos molluszka faj héjmaradványait vizsgáltuk, ezek közül négy tengeri csigafaj (*Bufonaria echinata *(Link, 1807),* Tibia fusus *(Linnaeus, 1758),* Architectonica perspectiva *(Linnaeus, 1758),* Cypraea turdus winckworthi *(F. A. Schilder & M. Schilder, 1938)) és négy kagyló faj (*Vepricardium asiaticum *(Bruguière, 1789),* Callista umbonella *(Lamarck, 1818),* Saccostrea cucullata *(Born, 1778),* Anodonta cygnea *(Linnaeus, 1758)), amelyeket természetes élőhelyükről gyűjtöttünk be vizsgálatra 2020-ban. Mikro-Raman spektroszkópiát használtunk a héjak külső, aragonit rétegének azonosítására, amely a szklerokémiai kutatásokhoz szükséges. Továbbá, szklerokronológiai módszerekkel vizsgáltuk, hogy a molluszka héjak felhasználhatók-e ilyen célú kutatásokhoz. A Raman spektroszkópiai vizsgálatok mindegyik taxon esetében kimutatták az aragonit jelenlétét a* Saccostrea cucullata *kivételével. Valamennyi héjmaradványon megfigyelhető volt a pigmentek jelenléte, kivéve a* Callista umbonella *taxont. A héjak keresztmetszetének vizsgálata szerint a* Callista umbonella *héjak jól megfigyelhető napi, havi és*

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éves növekedési vonalakat mutatnak, amelyek a külső rétegben elérik a 1650 µm vastagságot. Mindezek alapján Callista umbonella *héjai várhatóan jól használhatók a szklerokronokémiai vizsgálatokhoz.*

KEYWORDS: MOLLUSK SHELL, BIVALVE, SEA SNAIL, ARAGONITE, RAMAN SPECTROSCOPY, SCLEROCHRONOLOGY, *CALLISTA UMBONELLA*

KULCSSZAVAK: MOLLUSZKA HÉJ, KAGYLÓ, TENGERI CSIGA, ARAGONIT, RAMAN SPEKTROSZKÓPIA, SZKLEROKRONOLÓGIA, *CALLISTA UMBONELLA*

Introduction

Mollusks are a phylum of invertebrates including gastropods and bivalves, whose soft bodies are protected by a shell of calcium carbonate (Leng & Lewis 2016). They live in saltwater, freshwater, and terrestrial habitats (Antczak & Cipriani 2008; Andrus 2011) and serve as a source of food and decoration for humans. Mollusk shells tend to remain well preserved in sediments and are often abundantly found in archaeological sites (Leng & Lewis 2016). In the Persian Gulf region, a significant number of these specimens have been observed in the construction materials of historical buildings as well as archaeological excavations (Ghaderi & Darabi 2021).

Because of the key role of ancient shells and shell fossils in the reconstruction of past climate conditions, they have long been a matter of interest to geological (Epstein & Lowenstam 1953; Vernal et al. 1992; Geary et al. 1992; Goodwin et al. 2001; Ullmann et al. 2017; Killam & Clapham 2018; Scholz et al. 2020; Morán et al. 2021), archaeological studies (Quitmyer et al. 1997; Leng & Lewis 2016). Indeed, one of the main motivations for the study of bivalves (Müller et al. 2017; Huyghe et al. 2020; Das et al. 2021; Kelemen et al. 2021;), gastropods (Cespuglio et al. 1999; García-Escárzaga et al. 2019; Irie & Suzuki 2020), and extinct species such as belemnites (Epstein et al. 1951; Urey et al. 1951; Epstein et al. 1953; Alberti et al. 2021) is to gain insight into climatic conditions.

In general, many marine mollusks grow their shell or exoskeleton from the chemical compounds contained in seawater. These shells grow in distinct bands (like tree rings) along an axis of growth. More bands are added to the shell as the organism grows and becomes larger. The environmental conditions of the habitat tend to be reflected in the types of isotopes present in the shell. In other words, the shell contains geochemical signatures reflecting the habitat and life of the mollusk. Most importantly, stable isotopes of oxygen in seawater are stored in the carbonate of these shells (Dole 1952; Cooper et al. 1991; Aravena et al. 1993; Scholz et al. 2020). This process, which is called bio-mineralization, provides a chemical signature of seawater at the time of shell formation (Vert et al. 2012). The analysis of these shells helps archaeologists to reconstruct past environments and living conditions in and around marine sites and understand how people in these areas lived and worked in the past (Salisbury 2022). Since significant numbers of these shells can be found in archaeological sites, they can offer valuable insights into the living conditions of the organism. This insight can be gained by analyzing the stable isotopes in the shell in order to estimate the environmental conditions under which the organism has been living. In general, isotopes of oxygen and carbon provide excellent evidence for the study of climatic changes.

These characteristics of shells have led to the formation of research fields under titles such as sclerochronology, sclerochemistry and sclerochronochemistry. Sclerochronology is the study of periodic physical and chemical variations in the accretionary hard tissues of organisms, and the temporal context in which they formed (Jones et al. 2007; Estrella-Martinez 2019; Moss et al. 2021). But it has been proposed to use sclerochronology for studies of the physical structure of the hard tissues of organisms, even when combined with geochemistry (e.g. growth-line periodicity), and that sclerochemistry, as a sub-discipline of sclerochronology, be used to describe solely geochemical (isotopic or elemental) studies of the hard tissues of organisms. Whether these terms and distinctions are adopted or not, it does provide an essential difference between the two approaches (Gröcke & Gillikin 2008). However, it is possible that the established practice in part of terminology within sclerochronology communities might inhibit the navigation in literature, paper preparation and lead to confusion among new researchers. Therefore, the new term, ‘sclerochronochemistry’ (skleros – hard, chronos – time, and chemistry) has been proposed, in order to fill a gap between two key terms, sclerochronology and sclerochemistry (Zuykov & Schindler 2019).

The exoskeletons of aquatic invertebrates (mollusks, cephalopods, foraminifers, brachiopods) are made of calcium carbonates that are bio-mineralized in the form of aragonite or calcite (Lécuyer et al. 2012). Research has shown that in mollusks with aragonite shells, the $\delta^{18}O$ of the shell is almost or completely in equilibrium with that of seawater (Leng & Lewis 2016). In other words, the amount of oxygen isotopes in aragonite shells is independent of physiological fractionation processes and life conditions of the individual organism (Epstein et al. 1951; Epstein et al. 1953;

Epstein & Lowenstam 1953; Anderson & Arthur 1983; Grossman & Ku 1986; Quitmyer et al. 1997). Therefore, more reliable information about climatic changes can be obtained from the study of stable isotopes in aragonite shells than in calcite shells. Thus, it is important to identify the type of a shell before examining its isotope ratios.

There are various methods to identify and distinguish calcite and aragonite shells. The most common methods for recognizing aragonite shells are SEM and XRD (Gutiérrez-Zugasti et al. 2015). However, Raman spectroscopy is also a reliable method for identifying aragonite shells or detecting aragonite phases in an ancient or modern shell (Scholz et al. 2020). Raman spectroscopy can also be used to detect the vaterite phase, which is less stable than aragonite (Zhou et al. 2010).

Another important issue in sclerochronochemical examinations, besides the type of shell, is to collect the sample from the right position. The microstructural morphology of the shells varies depending on the species. In general, shells are composed of an inner layer and an outer layer. In *Mercenaria stimpsoni* (Gould 1861), for example, the shell consists of an outer layer composed of prismatic and lamellar microstructures and an inner layer with a homogeneous microstructure. However, $\delta^{18}O$ can only be measured on the prismatic outer layer (Kubota et al. 2017). Because the growth bands are seen in this layer and the entry of powder samples from the lower layers disrupts the calculations. Also, because of the low thickness of the prismatic layer in the early stages of life, younger species are not very suitable for sampling (Kubota et al. 2017). In some cases (some sea snails for example), the aragonite layer is coated with a layer of calcite, which must be removed before the inner aragonite layer can be sampled (García-Escárzaga et al. 2019). Therefore, all sclerochronology, sclerochemistry, and sclerochronochemistry studies should only be done with a good understanding of the microstructure, morphology, and chemistry of the shell.

The oxygen isotope analysis of mollusk shells allows past environmental conditions to be estimated through quantitative measurements (Twaddle et al. 2016; Mau et al. 2021). To do so, first, the relationship between the mollusk species and its habitat needs to be studied. For the quantitative measurement of past environmental conditions and climatic changes based on $\delta^{18}O$ in ancient shells, it is necessary to measure the isotope composition in present proxies including the present shells, water, and atmosphere. This technique is known as the transfer function approach (Veron 2011; Leng & Lewis 2016). Once this transfer function is determined for the present habitat-shell relationship, assuming that this relationship has not changed over time, it can also be applied to ancient specimens. Using this approach, one can estimate the temperature change quantitatively in terms of degrees instead of expressing it qualitatively by stating that the climate has become warmer or colder (Emiliani et al. 1964; Patterson et al. 2010). Therefore, in sclerochronology studies, it is also important to choose suitable present-day specimens for oxygen isotope analyses.

As explained above, the first step of sclerochemistry and sclerochronochemistry studies is to identify the constituent phases of the mollusk shell. However, the shell microstructure should also be suitable enough for sampling and examination of growth lines (sclerochronology examinations). In fact, growth lines should be clear in prismatic layers for sampling different growth times. In other words, in order to use a shell specimen to study changes in the ratio of stable oxygen isotopes during the mollusk lifetime, it should have examinable growth lines as well as an aragonite structure with a thick enough outer layer, so that sampling can be done without interference of the layers. In this paper, micro Raman spectroscopy and microscopic and sclerochronological methods were used to examine shells and aragonite and calcite phases of two-layered shells (shells with an inner layer and an outer layer) of common mollusk species of northern shores of Persian Gulf and identify prismatic layers suitable for sampling in these species.

Materials and methods

Specimens

The specimens were from eight common native species of the Strait of Hormuz and the Persian Gulf, including four sea snails (*Bufonaria echinata* (Link, 1807), *Tibia fusus* (Linnaeus, 1758), *Architectonica perspectiva* (Linnaeus, 1758), *Cypraea turdus winckworthi* (F. A. Schilder & M. Schilder, 1938)) and four bivalves (*Vepricardium asiaticum* (Bruguière, 1789), *Callista umbonella* (Lamarck, 1818), *Saccostrea cucullata* (Born, 1778), *Anodonta cygnea* (Linnaeus, 1758)) (**Fig. 1.**).

The specimens were randomly collected from the coastal areas of Bushehr, Qeshm Island, and Bostanu in 2020 (**Table 1.**). The location of specimen collection sites on the map is shown in **Fig. 2**. Considering that *Anodonta cygnea* is a species of freshwater mussel, it may have entered the coastal area from the rivers discharging into the Persian Gulf. Several specimens of each shell were randomly collected to evaluate the probability of error. After transferring the specimens to the laboratory, the meat tissue was removed, and the shells were washed with distilled water.

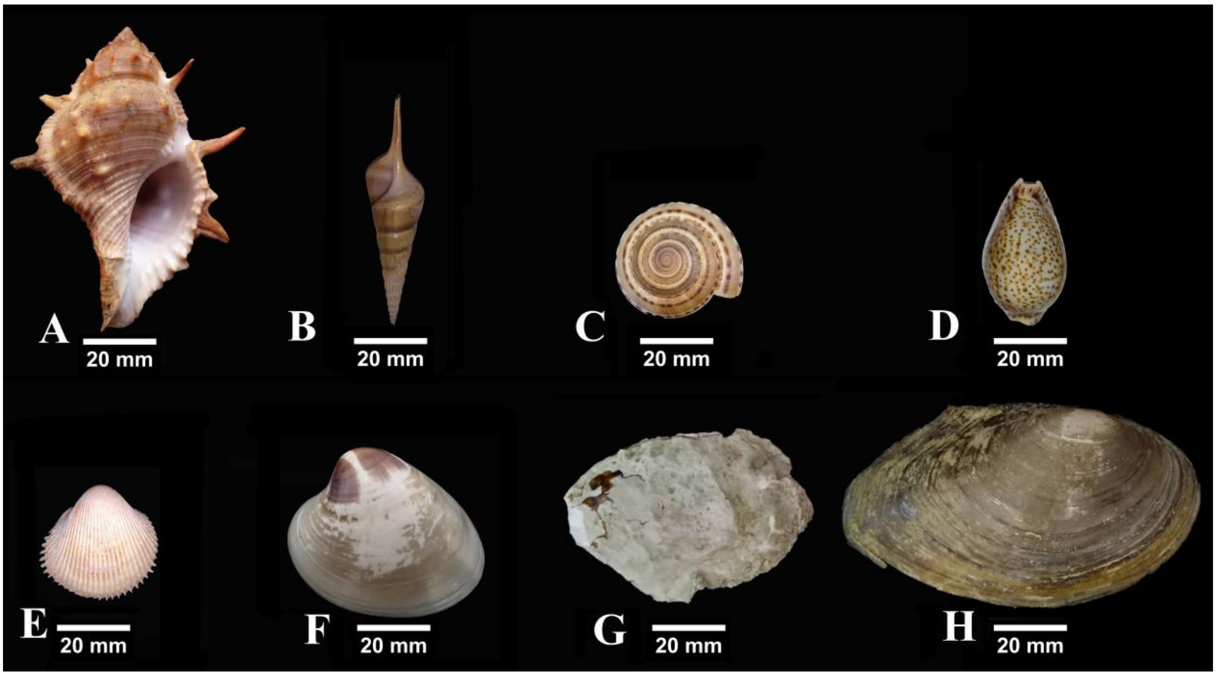

Fig. 1.: Studied specimens - sea snails (top row): A) *Bufonaria echinata*, B) *Tibia fusus*, C) *Architectonica perspectiva*, D) *Cypraea turdus winckworthi*; bivalves (bottom row): E) *Vepricardium asiaticum*, F) *Callista umbonella*, G) *Saccostrea cucullata*, H) *Anodonta cygnea*

1. ábra: A vizsgált fajok - tengeri csigák (felső sor): A) *Bufonaria echinata*, B) *Tibia fusus*, C) *Architectonica perspectiva*, D) *Cypraea turdus winckworthi*; kagylók (alsó sor): E) *Vepricardium asiaticum*, F) *Callista umbonella*, G) *Saccostrea cucullata*, H) *Anodonta cygnea*

Table 1.: Studied species, specimen collection sites and dates

1. táblázat: A vizsgált fajok, a mintagyűjtés helye és időpontja

No. of specimens	Date of catch	Shellfishing locations	Species
2	August, 2020	Bandar Bushehr	*Bufonaria echinata*
2	August, 2020	Bandar Bushehr	*Tibia fusus*
3	August, 2020	Bandar Bushehr	*Architectonica perspectiva*
3	August, 2020	Bandar Bushehr	*Cypraea turdus winckworthi*
2	August, 2020	Bandar Bushehr	*Vepricardium asiaticum*
10	September, 2020	Bostanu, Hormozgan	*Callista umbonella*
1	September, 2020	Qeshm island	*Saccostrea cucullata*
3	September, 2020	Qeshm island	*Anodonta cygnea*

A number of collected bivalves was separated for further examination. The shells were immersed in 10% hydrogen peroxide (Merck, Germany) for 3 hours to eliminate organic contaminations and then washed once with distilled water and two times in succession with 99% methanol (Merck, Germany). After drying, the outer and inner surfaces of the shells were examined for the presence of aragonite and their inner morphology was studied.

Micro Raman spectroscopy

Raman spectroscopy was performed using a Tekram Raman microscope (Teksan, Iran) with 532 nm laser excitation. The spectra were recorded in the 100–4000 cm^{-1} range with a resolution of 6 cm^{-1} and the results of five 5-second scans were averaged. Analysis was performed with a 10x objective lens at 40% laser power. Spectroscopy was conducted on both inner and outer surfaces of the shells.

Fig. 2.: Geographical location of specimens' collection sites in Persian Gulf, including coastal areas of Bushehr, Bostanu and Qeshm island. Satellite imagery provided by Google Earth

2. ábra: A vizsgált minták gyűjtési helye a Perzsa-öbölben, Bushehr, Bostanu és Qeshm szigetek parti régióinak kiemelésével (Google Earth műholdas felvételei alapján)

For *Architectonica perspectiva*, which had different color tonalities on its outer surface, both white and brown parts of the species were analyzed. The spectra were processed in the software Originpro2021. The baseline correction was performed in the 100–2000 cm^{-1} range.

Internal morphology

To study the internal morphology of the shells, they were cut to examine their cross-sections. Before cutting the specimens, a 1–2 mm thick layer of epoxy adhesive was applied around the cutting site. Bivalve specimens were cut along the longest path from the umbo to the ventral margin using a micro-cutter with a blade thickness of 1 mm. Snail specimens were cut at their largest diameters. After cutting the excess part of the shell to a thickness of 2–3 mm, sanding and polishing were performed. Once sanded and polished, the cut section was glued to a laboratory slide using epoxy adhesive. After preparing the sections, microscopy was performed with a Leica MS5 stereomicroscope equipped with two fiber-optic light sources with a 45º angle to the section and a Canon camera. The specimens were examined at 40x magnification and an average of 300 images were taken from each specimen. The microscopic images were integrated using the software Adobe Photoshop 2021.

Results and discussion

The results of micro Raman spectroscopy of the inner and outer surfaces of the shells under 532 nm excitation are presented in **Fig. 3**. Aragonite can be recognized by a distinct band around 1080 cm^{-1} related to the symmetric stretching vibrations of CO_3^{2-} and the bands around 700 cm^{-1} (in-plane bending vibrations), 200 cm^{-1}, and 148 cm^{-1}.

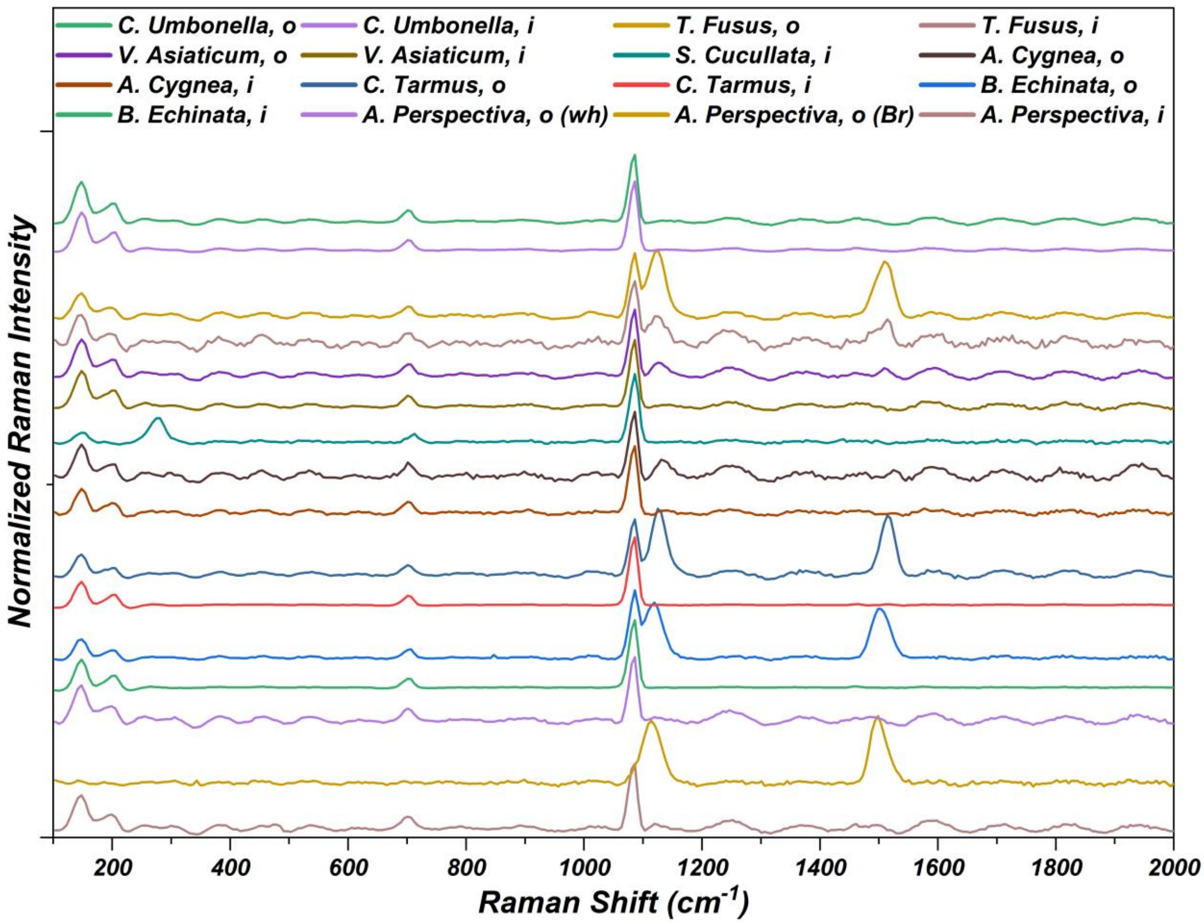

Fig. 3.: Micro Raman spectra of the inner and outer surfaces of the studied shells under 532 nm laser excitation; For the outer surface of *Architectonica perspectiva*, white and brown parts were analyzed

3. ábra: A vizsgált héjak külső és belső felszínének spektrumai 532 nm-es mikro-Raman lézer sugárzással vizsgálva. Az *Architectonica perspectiva* külső felszíne esetében a fehér és a barna részeket vizsgáltuk.

For calcite, however, the band related to the symmetric stretching vibrations of CO_3^{2-} appears around 1085 cm^{-1}, the band related to the in-plane bending vibrations appears around 714 cm^{-1}, and the bands related to the external vibrations of the CO_3 group appear around 150 and 280 cm^{-1} (Tomić et al. 2010).

The bands identified in the Raman spectra of the specimens are listed in **Table 2**. Examination of these spectra showed that only the shell of *Saccostrea cucullata* has a calcite structure. In this shell, calcite vibrations appear at 1086, 712, 278, and 151 cm^{-1}. In other specimens, the inner and outer surfaces of the shells were mostly made of aragonite. However, the spectra of the outer surface of all shells except *Callista umbonella* showed some bands in the 1100–1150 cm^{-1} and 1490–1530 cm^{-1} range, which are usually attributed to the vibrations of polyene pigments contained in the shells (Komura et al. 2018). Because of the accumulation of polyene pigments in the brown parts of the outer surface of the shell of *Architectonica perspectiva*, the spectra of these parts only showed the characteristic bands of polyene and did not reveal any aragonite vibration. However, there is no previous report of this interfering effect of the presence of polyene in the oxygen isotope analysis of mollusk shells. Since no substance other than aragonite was detected on the inner and outer surfaces of the shell of *Callista umbonella*, this species can be considered the superior choice for sclerochronology and sclerochemistry studies.

Selection of mollusk species for sclerochronology and sclerochemistry studies requires careful consideration of the morphological characteristics and the section of the shell being analyzed. The outer layer of bivalve shells contains a prismatic section, which is the only suitable site for $\delta^{18}O$ measurements. Identifying the outer prismatic layer and ensuring the thickness and visibility of the growth lines are vital for reliable sampling. So, merely detecting aragonite in the shell is not sufficient for species selection. Therefore, the cross-section of the studied shells was examined.

Table 2.: Wavenumbers of bands observed in the spectroscopy of the shell specimens under 532 nm excitation
2. táblázat: 532 nm-es lézersugárral vizsgált csiga- és kagylóhéj minták hullámszám értékei

Analyzed location	Species	Raman shifts of representative band (cm^{-1})									
		Aragonite				Calcite				Polyene Pigments	
Bufonaria echinata	Inner surface	1084	703	202	147	-	-	-	-	-	-
	Outer surface	1086	706	201	147	-	-	-	-	1119	1502
Tibia fusus	Inner surface	1085	707	201	147	-	-	-	-	1128	1516
	Outer surface	1085	706	203	149	-	-	-	-	1125	1511
Archi-tectonica perspectiva	Inner surface	1084	703	196	146	-	-	-	-	-	-
	Outer white surface	1084	702	196	146	-	-	-	-	-	-
	Outer brown surface	-	-	-	-	-	-	-	-	1113	1498
Cypraea turdus winckworthi	Inner surface	1084	703	202	147	-	-	-	-	1125	1515
	Outer surface	1084	702	201	147	-	-	-	-	1127	1515
Vepricardium asiaticum	Inner surface	1085	702	201	148	-	-	-	-	-	-
	Outer surface	1085	704	201	148	-	-	-	-	1127	1509
Callista umbonella	Inner surface	1085	704	203	149	-	-	-	-	-	-
	Outer surface	1084	705	203	148	-	-	-	-	-	-
Saccostrea cucullata	Inner surface	-	-	-	-	1086	712	278	151	-	-
Anodonta cygnea	Inner surface	1084	703	199	148	-	-	-	-	-	-
	Outer surface	1085	702	200	147	-	-	-	-	1132	1525

The examined sections are shown in **Fig. 4.** and the average thickness of the prismatic layer is given in **Table 3**. Because of the very low thickness of the shell of *Architectonica perspectiva*, it was not possible to prepare a cross-section from this shell. In the cross-section of the shell of *Saccostrea cuccullata*, which had a calcite structure, the prismatic part and the outer layer were somewhat indistinguishable. Although well identifiable, the prismatic parts of the shells of *Bufonaria echinata*, *Tibia fusus*, *Vepricardium asiaticum*, and *Anodonta cygnea* were less than 500 µm thick. This low thickness makes the sampling more difficult and increases the probability of error. In other words, it increases the likelihood that parts of the shell's inner layer are removed along with the sample, which can cause an error in the analysis results. While the outer layer of the shell of *Cypraea turdus winckworthi* had an average thickness of about 830 µm, the thickness was concentrated in a small part of the shell, making it impossible to take a sample from the entire outer surface.

The average thickness of the outer layer of the shell of *Callista umbonella* was about 1650 µm, making it easier to sample for the analysis of stable oxygen isotope ratios. In fact, considering the aragonite structure of its inner and outer layers as well as the appropriate thickness of the outer layer, the shell of *Callista umbonella* can be considered an excellent choice for sclerochemistry studies. **Fig. 5.** shows the microscopic image of the cross-section of this shell along with some extra details. The daily, monthly (tidal) and annual growth lines are well distinguishable in this cross-section. Thanks to these features, *Callista umbonella* is not only suitable for sclerochemistry studies, but can also be a valuable asset for sclerochronology and sclero-chronochemistry research.

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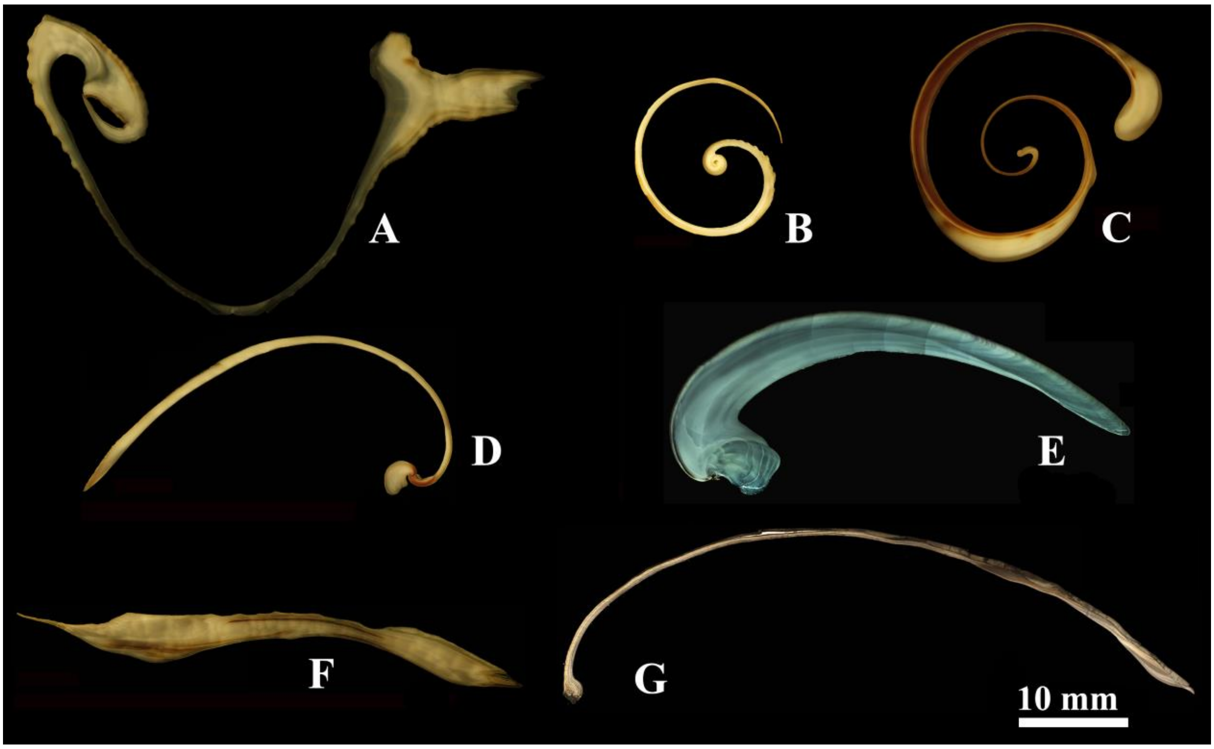

Fig. 4.: Microscopic images of the cross-sections of the studied shells: A) *Bufonaria echinata*, B) *Tibia fusus*, C) *Cypraea turdus winckworthi*, D) *Vepricardium asiaticum*, E) *Callista umbonella*, F) *Saccostrea cucullata*, G) *Anodonta cygnea*

4. ábra: A vizsgált puhatestű héjak keresztmetszeteinek mikroszkópi képe: A) *Bufonaria echinata*, B) *Tibia fusus*, C) *Cypraea turdus winckworthi*, D) *Vepricardium asiaticum*, E) *Callista umbonella*, F) *Saccostrea cucullata*, G) *Anodonta cygnea*

Table 3.: Average thickness of the outer layer in shells suitable for sampling for oxygen isotope analyses

3. táblázat: A héjak külső, oxigén izotópos vizsgálatra alkalmas rétegének átlagos vastagságai

Species	Average thickness of the outer layer (µm)
Bufonaria echinata	440
Tibia fusus	430
Architectonica perspectiva	Preparation failed
Cypraea turdus winckworthi	830
Vepricardium asiaticum	500
Callista umbonella	1732
Saccostrea cucullata	Not detected
Anodonta cygnea	420

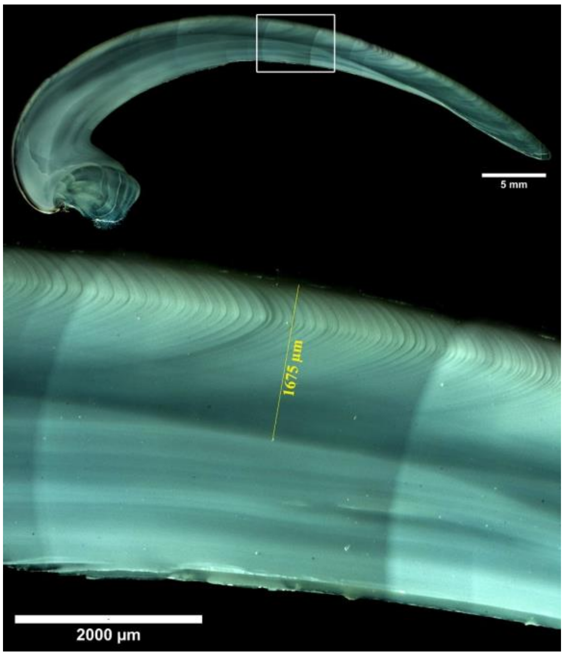

Fig. 5.: Microscopic image of the cross-section of the *Callista umbonella* shell

5. ábra: A *Callista umbonella* héjának mikroszkópi képe (keresztmetszet)

Conclusion

Shells make up a significant portion of materials obtained from some archaeological sites. Studying these shells as an archaeological record can help researchers reconstruct past climatic conditions. However, for quantitative estimation of past climates, researchers have to study not only ancient specimens but also present-day shells to determine the relationship between shell structure and its growth environment in order to formulate a transfer function. For a shell to be considered suitable for sclerochronology and sclerochemistry studies, it must be made of aragonite and have the right conditions for sampling.

In this study, the shells of eight common native species of the northern shores of the Persian Gulf were examined to determine whether they possess these features. Micro Raman spectroscopy of the inner and outer layers of the shells showed aragonite structure in all shells except *Saccostrea cucullata*. Considering the bands observed in its Raman spectrum, the shell of *Saccostrea cucullata* has a calcite structure, which precludes the use of this shell in oxygen isotope analyses.

Vibrations of polyene pigments around 1120 cm^{-1} and 1500 cm^{-1} were observed in the Raman spectrum of the outer surface of all shells except the white shell of *Callista umbonella*. Thus, considering the fully aragonite structure of the inner and outer layers of the shell of this mollusk and the lack of polyene interference in its Raman spectrum, this species can be considered the superior choice for sclerochemistry studies. Cross-sectional examinations also showed that, with a thickness of 1650 µm in its outer layer, the shell of *Callista umbonella* is the best choice among the considered options for sampling for oxygen isotope analyses. The presence of clearly distinguishable daily, monthly and annual growth lines in this shell also makes it suitable for sclerochronology and sclerochronochemistry studies.

Contribution of authors

Alireza Koochakzaei Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – Original draft, Visualization, Writing – Review and Editing. **Salar Yazdanbakhshi** Investigation, Resources, Writing – Original draft, Visualization. **Mohsen Mohammadi Achachlouei** Resources, Writing – Original draft.

References

ALBERTI, M., FÜRSICH, F.T., PANDEY, D.K., ANDERSEN, N., GARBE-SCHÖNBERG, D., BHOSALE, S., CHASKAR, K. & HABERMANN, J.M. (2021): First record of stable isotopes ($\delta^{13}C$, $\delta^{18}O$) and element ratios (Mg/Ca, Sr/Ca) of Middle to Late Jurassic belemnites from the Indian Himalayas and their potential for palaeo-environmental reconstructions. *Journal of Palaeogeography* **10/24** 1-22 https://doi.org/10.1186/s42501-021-00103-2

ANDERSON, T.F. & ARTHUR, M.A. (1983): Stable Isotopes of Oxygen and Carbon and their Application to Sedimentologic and Paleoenvironmental Problems. In: Arthur, M.A., Anderson, T.F., Kaplan, I.R., Veizer, J. & Land, L.S. (eds.), *Stable Isotopes in Sedimentary Geology* (Vol. **10,** 1–151): SEPM Society for Sedimentary Geology. https://doi.org/10.2110/scn.83.01.0000

ANDRUS, C.F.T. (2011): Shell midden sclerochronology. Quaternary Scientific Review **30/21-22** 2892–2905. https://doi.org/10.1016/j.quascirev.2011.07.016

ANTCZAK, A.T. & CIPRIANI, R. (2008): *Early Human Impact on Megamolluscs*. Archaeopress. Oxford, England, 1-5.

ARAVENA, R., EVANS, M.L. & CHERRY, J.A. (1993): Stable Isotopes of Oxygen and Nitrogen in Source Identification of Nitrate from Septic Systems. *Groundwater* **31/2** 180–186. https://doi.org/10.1111/j.1745-6584.1993.tb01809.x

CESPUGLIO, G., PICCINETTI, C. & LONGINELLI, A. (1999): Oxygen and carbon isotope profiles from *Nassa mutabilis* shells (Gastropoda): accretion rates and biological behaviour. Marine Biology **135** 627–634. https://doi.org/10.1007/s002270050663

COOPER, L.W., OLSEN, C.R., SOLOMON, D.K., LARSEN, I.L., COOK, R.B. & GREBMEIER, J.M. (1991): Stable Isotopes of Oxygen and Natural and Fallout Radionuclides Used for Tracing Runoff During Snowmelt in an Arctic Watershed. *Water Resources Research* **27/9** 2171–2179. https://doi.org/10.1029/91wr01243

DAS, S., JUDD, E.J., UVEGES, B.T., IVANY, L.C. & JUNIUM, C.K. (2021): Variation in $\delta^{15}N$ from shell-associated organic matter in bivalves: Implications for studies of modern and fossil ecosystems. *Palaeogeography Palaeoclimatology Palaeoecology* **562** 110076. https://doi.org/10.1016/j.palaeo.2020.110076

DOLE, M. (1952): The Chemistry of the Isotopes of Oxygen. *Chemical Reviews* **51/2** 263–300. https://doi.org/10.1021/cr60159a002

EMILIANI, C., CARDINI, L. & MAYEDA, T. (1964): Paleotemperature analysis of fossil shells of marine mollusks (food refuse) from the Arene Candide cave, Italy and the Haua Fteah cave, Cyrenaica. *Isotopic Cosmic Chemistry*, **16** 133–156.

EPSTEIN, S., BUCHSBAUM, R., LOWENSTAM, H. & UREY, H.C. (1951): Carbonate-water isotopic temperature scale. *Geological Society of America Bulletin* **62/4** 417–426. https://doi.org/10.1130/0016-7606(1951)62[417:CITS]2.0.CO;2.

EPSTEIN, S., BUCHSBAUM, R., LOWENSTAM, H.A., & UREY, H.C. (1953): Revised carbonate-water isotopic temperature scale. *Geological Society of America Bulletin* **64/11** 1315–1326. https://doi.org/10.1130/0016-7606(1953)64[1315:RCITS]2.0.CO;2

EPSTEIN, S. & LOWENSTAM, H.A. (1953): Temperature-Shell-Growth Relations of Recent and Interglacial Pleistocene Shoal-Water Biota from Bermuda. *Journal of Geology* **61** 424–438. https://doi.org/10.1086/626110

ESTRELLA-MARTINEZ, J. (2019): Holocene Climate Variability in UK Waters Based on Arctica islandica Sclerochronology. Bangor University (United Kingdom). Ph.D. thesis, 141 pp.

GARCÍA-ESCÁRZAGA, A., GUTIÉRREZ-ZUGASTI, I., COBO, A., SOLANA, D.C., CHIVELET, J.M., Roberts, P. & GONZÁLEZ-MORALES M.R. (2019): Stable oxygen isotope analysis of *Phorcus lineatus* (da Costa, 1778) as a proxy for foraging seasonality during the Mesolithic in northern Iberia. *Archaeological and Anthropological Sciences* **11** 5631–5644. https://doi.org/10.1007/s12520-019-00880-x

GEARY, D.H., BRIESKE, T.A. & BEMIS, B.E. (1992): The influence and interaction of temperature, salinity, and upwelling on the stable isotopic profiles of strombid gastropod shells. *PALAIOS*. **7/1** 77–85. https://doi.org/10.2307/3514797

GHADERI, H. & DARABI, H. (2021): A Survey of Origin and Production of the PPN Shell Beads from Ali Kosh, Deh Luran Plain. *Journal of Archaeological Studies* **13/3** 193–210. https://doi.org/10.22059/jarcs.2020.298646.142856

GOODWIN, D.H., FLESSA, K.W., SCHÖNE, B.R. & DETTMAN, D.L. (2001): Cross-Calibration of Daily Growth Increments, Stable Isotope Variation, and Temperature in the Gulf of California Bivalve Mollusk Chione cortezi: Implications for Paleoenvironmental Analysis. *PALAIOS*. **16/4** 387–398. https://doi.org/10.1669/0883-1351(2001)016<0387:CCODGI>2.0.CO;2

GRÖCKE, D.R. & GILLIKIN, D.P. (2008): Advances in mollusc sclerochronology and sclerochemistry: tools for understanding climate and environment. *Geo-Marine Letters* **28** 265–268. https://doi.org/10.1007/s00367-008-0108-4

GROSSMAN, E.L. & KU, T.L. (1986): Oxygen and carbon isotope fractionation in biogenic aragonite: Temperature effects. *Chemical Geology: Isotope Geoscience section* **59** 59–74. https://doi.org/10.1016/0168-9622(86)90057-6

GUTIÉRREZ-ZUGASTI, I., GARCÍA-ESCÁRZAGA, A., MARTÍN-CHIVELET, J. & GONZÁLEZ-MORALES, M.R. (2015): Determination of sea surface temperatures using oxygen isotope ratios from *Phorcus lineatus* (Da Costa, 1778) in northern Spain: Implications for paleoclimate and archaeological studies. *The Holocene* **25/6** 1002–1014. https://doi.org/10.1177/0959683615574892

HUYGHE, D., EMMANUEL, L., DE RAFELIS, M., RENARD, M., ROPERT, M., LABOURDETTE, N. & LARTAUD, F. (2020): Oxygen isotope disequilibrium in the juvenile portion of oyster shells biases seawater temperature reconstructions. *Estuarine, Coastal and Shelf Science* **240** 106777. https://doi.org/10.1016/j.ecss.2020.106777

IRIE, T. & SUZUKI, A. (2020): High temperature stress does not distort the geochemical thermometers based on biogenic calcium carbonate: Stable oxygen isotope values and Sr/Ca ratios of gastropod shells in response to rearing temperature. *Geochimica et Cosmochimica Acta* **288** 1–15. https://doi.org/10.1016/j.gca.2020.07.044

JONES, D.S., ARNOLD, B., QUITMYER, I., SCHÖNE, B.R. & SURGE, D. (2007): 1st International Sclerochronology Conference. http://conference.ifas.ufl.edu/sclerochronology. Accessed 10 April 2020. July 17-21. St. Petersburg, Florida, USA.

KELEMEN, Z., GILLIKIN, D.P., BORGES, A.V., TAMBWE, E., SEMBAITO, A.T., MAMBO, T., WABAKHANGAZI, J.N., YAMBÉLÉ, A., STROOBANDT, Y. & BOUILLON, S. (2021): Freshwater bivalve shells as hydrologic archives in the Congo Basin. *Geochimica et Cosmochimica Acta* **308** 101–111. https://doi.org/10.1016/j.gca.2021.05.023

KILLAM, D.E. & CLAPHAM, M.E. (2018): Identifying the ticks of bivalve shell clocks: seasonal growth in relation to temperature and food supply. *PALAIOS*. **33/5** 228–236. https://doi.org/10.2110/palo.2017.072

KOMURA, T., KAGI, H., ISHIKAWA, M., YASUI, M. & SASAKI, T. (2018): Spectroscopic Investigation of Shell Pigments from the Family Neritidae (Mollusca: Gastropoda). In: Endo, K., Kogure, T., Nagasawa, H. (eds) *Biomineralization*. Springer, Singapore, 73-82. https://doi.org/10.1007/978-981-13-1002-7_8

KUBOTA, K., SHIRAI, K., MURAKAMI-SUGIHARA, N., SEIKE, K., MASAKO HORI, M. & TANABE, K. (2017): Annual shell growth pattern of the Stimpson's hard clam Mercenaria stimpsoni as revealed by sclerochronological and oxygen stable isotope measurements. *Palaeogeography Palaeoclimatology Palaeoecology* **465** 307–315. https://doi.org/10.1016/j.palaeo.2016.05.016

LÉCUYER, C., HUTZLER, A., AMIOT, R., DAUX, V., GROSHENY, D., OTERO, O., MARTINEAU, F., FOUREL, F., BALTER, V. & REYNARD, B. (2012): Carbon and oxygen isotope fractionations between aragonite and calcite of shells from modern molluscs. *Chemical Geology* **332-333** 92–101. https://doi.org/10.1016/j.chemgeo.2012.08.034

LENG, M.J. & LEWIS, J.P. (2016): Oxygen isotopes in Molluscan shell: Applications in environmental archaeology. *Environmental Archaeology* **21/3** 295–306. https://doi.org/10.1179/1749631414Y.0000000048

MAU, A., FRANKLIN, E.C., NAGASHIMA, K., HUSS, G.R., VALDEZ, A.R., NICODEMUS, P.N. & BINGHAM, J.-P. (2021): Near-daily reconstruction of tropical intertidal limpet life-history using secondary-ion mass spectrometry. *Communications Earth & Environment*, **2/1** 1–9. https://doi.org/10.1038/s43247-021-00251-2

MORÁN, G.A., BAYER, S., BEIERLEIN, L., MARTÍNEZ, J.J., BENITEZ-VIEYRA, S., MACKENSEN, A., BREY, T. & GORDILLO, S. (2021): Late Holocene Ameghinomya antiqua shells from the Beagle Channel: A multi-proxy approach to palaeoenvironmental and palaeoclimatic reconstruction. *Palaeogeography Palaeoclimatology Palaeoecology* **578** 110574. https://doi.org/10.1016/j.palaeo.2021.110574

MOSS, D.K., IVANY, L.C. & JONES, D.S. (2021): Fossil bivalves and the sclerochronological reawakening. *Paleobiology.* **47/4** 551–573. https://doi.org/10.1017/pab.2021.16

MÜLLER, P., STAUDIGEL, P.T., MURRAY, S.T. VERNET, R., BARUSSEAU, J.P., WESTPHAL, H. & SWART, P.K. (2017): Prehistoric cooking versus accurate palaeotemperature records in shell midden constituents. *Scientific Reports* **7** 3555. https://doi.org/10.1038/s41598-017-03715-8

PATTERSON, W.P., DIETRICH, K.A., HOLMDEN, C. & ANDREWS, J.T. (2010): Two millennia of North Atlantic seasonality and implications for Norse colonies. *The Proceedings of the National Academy of Sciences (PNAS)*. **107/12** 5306–5310. https://doi.org/10.1073/pnas.090252210

QUITMYER, I.R., JONES, D.S. & ARNOLD, W.S. (1997): The Sclerochronology of Hard Clams, Mercenaria spp., from the South-Eastern U.S.A.: A Method of Elucidating the Zooarchaeological Records of Seasonal Resource Procurement and Seasonality in Prehistoric Shell Middens. *Journal of Archaeological Science* **24/9** 825–840. https://doi.org/10.1006/jasc.1996.0163

SALISBURY, R.B. (2022): Introduction to Environmental Archaeology. STIMUL, Univerzita Komenského v Bratislave, Filozofická fakulta. 50–58.

SCHOLZ, S.R., PETERSEN, S.V., ESCOBAR, J. JARAMILLO, C., HENDY, A.J.W., ALLMON, W.D., CURTIS, J.H., ANDERSON, B.M., HOYOS, N., RESTREPO, J.C. & PEREZ, N. (2020): Isotope sclerochronology indicates enhanced seasonal precipitation in northern South America (Colombia) during the Mid-Miocene Climatic Optimum. *Geology.* **48/7** 668–672. https://doi.org/10.1130/G47235.1

TOMIĆ, Z., MAKRESKI, P. & GAJIĆ, B. (2010): Identification and spectra–structure determination of soil minerals: Raman study supported by IR spectroscopy and X-ray powder diffraction. *Journal of Raman Spectroscopy* **41/5** 582–586. https://doi.org/10.1002/jrs.2476

TWADDLE, R.W., ULM, S., HINTON, J., WURSTER, C.M. & BIRD, M.I. (2016): Sclerochronological analysis of archaeological mollusc assemblages: methods, applications and future prospects. *Archaeological and Anthropological Sciences.* **8** 359–379. https://doi.org/10.1007/s12520-015-0228-5

ULLMANN, C.V., FREI, R. & KORTE, C. & LÜTER, C. (2017): Element/Ca, C and O isotope ratios in modern brachiopods: Species-specific signals of biomineralization. *Chemical Geology* **460** 15–24 https://doi.org/10.1016/j.chemgeo.2017.03.034

UREY, H.C., LOWENSTAM, H.A., EPSTEIN, S. & McKINNEY, C.R. (1951): Measurement of Paleotemperatures and Temperatures of the Upper Cretaceous of England, Denmark, and the Southeastern United States. *Geological Society of America Bulletin* **62/4** 399–416. https://doi.org/10.1130/0016-7606(1951)62[399:mopato]2.0.co;2

VERNAL, A.D., BILODEAU, G., HILLAIRE-MARCEL, C. & KASSOU, N. (1992): Quantitative assessment of carbonate dissolution in marine sediments from foraminifer linings vs. shell ratios: Davis Strait, northwest North Atlantic. *Geology.* **20/6** 527–530. https://doi.org/10.1130/0091-7613(1992)020<0527:QAOCDI>2.3.CO;2

VERON, J.E.N. (2011): Scleractinia, evolution and taxonomy. In: Hopley, D. (eds): *Encyclopedia of Modern Coral Reefs.* Encyclopedia of Earth Sciences Series. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2639-2_78

VERT, M., DOI, Y., HELLWICH, K.H., HESS, M., HODGE, P., KUBISA, P., RINAUDO, M. & SCHUÉ, F. (2012): Terminology for biorelated polymers and applications (IUPAC Recommendations 2012). Pure and Applied Chemistry **84/2** 377–410.
https://doi.org/10.1351/PAC-REC-10-12-04

WEHRMEISTER, U., JACOB, D., SOLDATI, A., HÄGER, T. & HOFMEISTER, W. (2007): Vaterite in freshwater cultured pearls from China and Japan. *Journal of Gemmology* **31/5-6** 269–276.
https://doi.org/10.15506/JoG.2007.30.7.399

ZHOU, G.T., YAO, Q.Z., FU, S.Q. & GUAN, Y.B. (2010): Controlled crystallization of unstable vaterite with distinct morphologies and their polymorphic transition to stable calcite. *European Journal of Mineralogy* **22/2** 259–269.
https://doi.org/10.1127/0935-1221/2009/0022-2008

ZUYKOV, M. & SCHINDLER, M. (2019): Sclerochronology-based geochemical studies of bivalve shells: potential vs reality. *Estonian Journal of Earth Sciences.* **68/1** 37–44.
https://doi.org/10.3176/earth.2019.05