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The Mediterranean Sea during the Pleistocene - bivalve shells and their potential to reconstruct decadal and seasonal climate signals of the past

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Executive Summary

Understanding the climate of the past is essential for anticipating future climate change in marine ecosystems. Therefore, specifically, seasonal temperature amplitudes are of great concern. Understanding effects of changing seasonality in the geological past is important to predict and comprehend future transformations. The Mediterranean Sea is of particular importance because of its crucial role in modern atmosphere phenomena such as the North Atlantic Oscillation (NAO). Fossil shells of the bivalve *Arctica islandica* were collected from Pleistocene successions in Central and Southern Italy (i.e. Sicily, Rome and Lecce). According to preliminary data the studied deposits belong to the middle Calabrian between 1.2 and 0.9 Ma for the Sicily outcrop and 1.2 and 1.4 Ma for the Rome and Lecce outcrops, respectively. Cross-sections of *A. islandica* were prepared by cutting the shell along the line of strongest growth and grinding. Prior to isotope geochemical analysis confocal Raman microscopy measurements were conducted to detect potential diagenetic alterations (e.g. from aragonite to calcite). Stable oxygen isotope ($\delta^{18}O_{shell}$) samples were taken by micro-milling, measured with a continuous-flow isotope ratio mass spectrometer and used to reconstruct the seasonal water temperature amplitude. The $\delta^{18}O_{water}$ value was estimated to be 0.9±0.1‰ (V-SMOV). Stable oxygen isotope ($\delta^{18}O_{shell}$) results indicate a low seasonality scenario with an annual temperature variation of about 3°C and an average water temperature of 9-10°C in the middle Calabrian Mediterranean Sea. This is in sharp contrast to previous assumptions that the simultaneous occurrence of boreal (*A. islandica*) and warm-water species in the Mediterranean Sea during the Pleistocene can be explained by high seasonality (about 10°C). This suggests that the Sicilian outcrop represents a maximum glacial phase, which coincides with the high abundance of boreal species found in the outcrops. Measurements of annual growth increments in cross-sections reveal ontogenetic ages of up to 300 years. Derived standardized time-series are used for the identification of multi-year signals, which may be linked to large-scale climatic and atmospheric phenomena. Analysis by Singular Spectrum Analysis (SSA) and Multi-Taper Method (MTM) identified a prominent 6-year signal, which might be linked to the North Atlantic Oscillation (NAO) or the Mediterranean Oscillation (MO). The NAO has a 5-9 year periodicity and is often used to explain climate variability in Europe. The lack of a high seasonality and low mean water temperatures indicate that the middle Calabrian Mediterranean Sea was characterized by different climatic conditions compared to modern times.

Abstract

Understanding the climate of the past and past seasonal temperature amplitudes is essential to evaluate the effects of future climate change on marine ecosystems. The Mediterranean Sea is of great importance due to its crucial role in modern atmospheric phenomena such as the North Atlantic Oscillation (NAO). Fossil shells of the bivalve *Arctica islandica* were collected from three different Pleistocene successions in Italy. The seasonal water temperature amplitude was reconstructed using stable oxygen isotope ($\delta^{18}O_{shell}$) analysis. Samples were derived by the micro-milling approach and measured by isotope ratio mass spectrometer. Results show a low seasonality scenario (~3°C). This is in sharp contrast to the assumption that the simultaneous occurrence of boreal and warm-water species in the middle Calabrian Mediterranean Sea can be explained by high seasonality (~10°C). A prominent 6-year cyclicity was identified in the shell growth time-series by means of spectral analysis. This signal might be linked to the NAO whose periodicity ranges between 5-9 years. However, a connection to the Mediterranean Oscillation cannot be excluded. The low seasonality (~3°C) and the relatively low mean water temperature (9-10°C) indicate that the middle Calabrian Mediterranean Sea was characterized by colder climatic conditions compared to nowadays, indicating a maximum glacial phase.

1 Introduction

Understanding the climate of the past is essential to evaluate how marine ecosystems are affected by changing environmental conditions in the future. In particular, seasonal temperature amplitudes are of big concern. Understanding the impacts of changing seasonality during climate change in the geological past is important to predict and comprehend transformations which will also affect humans in the future (Crippa et al., 2016). Due to climate change it is expected that global water temperatures increase and lead to changing distribution patterns of marine species (IPCC, 2013). To predict the effects of global change we have to understand the natural long-term variability of environmental and climatic variables such as seasonality in the past.

Due to its crucial role in fisheries, being living habitat for millions of people and modern ocean atmosphere phenomena such as the North Atlantic Oscillation (NAO) and the El Niño-Southern Oscillation (ENSO) the Mediterranean Sea is of particular importance within the global change context (Giorgi and Lionello, 2008). Those phenomena are suggested to influence the winter rain variability over the Eastern Mediterranean (Giorgi and Lionello, 2008). Since the Mediterranean Sea is constituted as "one of the most important hot-spots in future climate change projections" (Giorgi, 2006) and underwent large climate shifts in the past (Luterbacher et al., 2006) it is of great importance within the global climate change context (Giorgi and Lionello, 2008). According to Bethoux et al. (1999) the Mediterranean Sea represents "a miniature ocean in relation to climate" and therefore it is well suited to understand global climatic patterns. Moreover, the Mediterranean Sea is a potentially vulnerable region to climate change (Giorgi and Lionello, 2008) explaining why it is important to understand how future climate change will affect this system.

By understanding the climate of the Pleistocene, which was dominated by dramatic climate and temperature shifts and their effects on Earth's biota, we are given a chance to better understand the effects of current and future global change. The Pleistocene covers the time span from 2.58 Ma to 11.7 ka covering four stages. One of these is the Calabrian comprising the time from 1.80 to 0.781 Ma (c.f. http://www.stratigraphy.org; v2016/04). The Pleistocene was the most recent episode of glacial cooling in which the temperate zone was relocated further south and the polar zone expanded (Eriscon and Wollin, 1968).

The development of large-scale Northern Hemisphere ice sheets caused a long-term cooling event coinciding with cooler surface water (Howell, 1990; Sosdian and Rosenthal, 2009). During the warmer Pliocene faunal assemblages in the Mediterranean were dominated by subtropical species (Thunell et al., 1991). The abundance of these warm-water species decreased significantly and the on-going cooling coincided with the migration of "boreal guests"

such as *Arctica islandica* in the Mediterranean Sea at around 1.7 to 1.6 Ma (Raffi, 1986; Nebout and Grazzini, 1991). The three successive floods of "boreal guests" migration correspond with glacial peaks of the southward migration of the polar front to mid latitudes of the North Atlantic. Close to marine isotope stage 62 (1.75 Ma) the Northern Hemisphere migration became severe enough to induce this expansion of the polar front (Nebout and Grazzini, 1991). Distribution patterns are limited by temperature ranges for reproduction as well as the survival of larvae (Raffi, 1986). Due to early Pleistocene climate changes the polar front migrated southwards and boreal guests entered the Mediterranean Sea by passing the Strait of Gibraltar (Nebout and Grazzini, 1991). However, subtropical taxa were found in faunal assemblages of *A. islandica* suggesting a simultaneous occurrence (Raffi, 1986). A high seasonality could explain the simultaneous occurrence of warm-water and boreal species (Raffi, 1986). Seasonality is a fundamental part of the climate system (Hansen et al., 2011). It has a big influence on biota and evolution including hominid evolution (Foley in Ulijaszek and Strickland, 1993). Fauna and flora will be affected by future climate change. Resolving seasonality during past climate changes implies a better understanding and predictability of future transformations. Previous studies, which used microfossils to determine palaeo-oceanographic parameters of the Mediterranean Sea, showed large-scale temperature oscillations on long time-series during the Pleistocene (Thunell, 1979a). Climate conditions of the Mediterranean Sea were extremely different in the middle Calabrian compared to today. *A. islandica* has gotten extinct in the Mediterranean Sea more than 9,800 years ago (Dahlgren et al., 2000). Its modern distribution in the temperate/boreal North Atlantic, where the species belongs to the largest bivalves, is limited southwards by high temperatures (Thórarinsdóttir and Einarsson, 1996). This is controlled by thermic requirements of gametogenesis preferring mean bottom water temperatures of ≤5-6°C (Raffi, 1986).

The ocean quahog *A. islandica* is a well-suited bio-archive of proxies that can reveal water temperature amplitude and multiyear signals, which are encoded in the anatomical, morphological and geochemical properties of the shell (Schöne, 2013). This bivalve is characterized by longevity (up to 507 years; Butler et al., 2013) and high resolution (daily increments; Schöne et al., 2005b) representing an ideal archive to understand past climatic (temperature) and ecological conditions (salinity). Moreover, water temperature reconstructions are based on the fact that *A. islandica* records the primary isotope composition of the seawater in which they lived with no vital effect (Hickson et al., 1999; Royer et al., 2013; Schöne, 2013). Stable oxygen isotopes ($\delta^{18}O_{shell}$) are a dual proxy providing information about water temperature and $\delta^{18}O_{water}$ (salinity). Changes in water temperature can be observed. However, absolute water temperatures can just be reconstructed if salinity values ($\delta^{18}O_{water}$) are known. Missing data on salinity and its variability in the geological past are a big challenge.

Consequently, $\delta^{18}O_{water}$ values have to be assumed according to palaeo-ecology or sediment core reconstructions (Crippa et al., 2016). Bivalve shells grow by incremental accretion of calcium carbonate ($CaCO_3$) (Schöne, 2013). Annual resolution is obtained by increments, which are defined as the growth between two annual growth lines representing one year. Since growth occurs during the warmest and coldest parts of the year seasonality is recorded in the shells (Schöne et al., 2004). Food availability and water temperature are the driving factors for shell growth. These factors, in turn, are influenced by climatic oscillations that indirectly trigger how much the shell grows. Frequency analysis of past shell growth time-series is based on this relation.

1.1 Aims and Objectives

This study mainly focuses on the variability of the seasonal water temperature amplitude in the Mediterranean Sea during the Pleistocene. I investigate this aspect by means of stable oxygen isotope measurements ($\delta^{18}O_{shell}$) within the shell carbonate of the bivalve *A. islandica*. Furthermore, I study multi-year climate signals within annual growth increments to help understand the climate of the past, which is in turn essential to anticipate future climate changes on marine ecosystems. In detail, the following issues and questions will be addressed and answered throughout this study:

- Do fossil *A. islandica* shells contain environmental information about the middle Calabrian (Pleistocene) and can those be encoded by means of stable oxygen isotope ($\delta^{18}O_{shell}$) measurements?

- Is high seasonality (~10°C) the explanation for the simultaneous occurrence of boreal (*A. islandica*) and warm-water species in the Pleistocene Mediterranean Sea?

- The arrival of boreal guests in the Mediterranean Sea occurred at around 1.8 Ma, indicating colder climate conditions. Besides the assumed seasonal variations, what was the average water temperature of the Mediterranean Sea during the middle Calabrian?

- Can fossil shells from the middle Calabrian be used for geochemical analysis ($\delta^{18}O_{shell}$) or does confocal Raman microscopy detect diagenetic alternations?

- Preferable environmental conditions favour shell growth and ontogenetic age of *A. islandica*. What is the maximum ontogenetic age of fossil Pleistocene *A. islandica* shells from Italy?

- Does frequency analysis reveal multi-year signals within the shell growth time-series, which can be linked to known modern day ocean-atmosphere phenomena (such as NAO)?

2   Material and methods

2.1   Geographical settings

In July 2015 molluscan and gastropod shells were collected at three different outcrops in Central and Southern Italy (Fig. 1). On July 14th, 2015 38 *A. islandica* shells, mainly umbos, and associated fauna were collected in an outcrop close to Rome (Lat 41.65°, Long 12.48°). This geological setting was characterized by silt and sand and was located close to the waterline of a flooded opencast (Fig. 2 A-C). Furthermore, on July 15th, 2015 8 *A. islandica* shells and umbos were collected in an old fossil park, Controfiano Park, Lecce, South Italy (Lat 40.13°, Long 18.18°). The shells were enclosed by sand (Fig. 2 D-F).
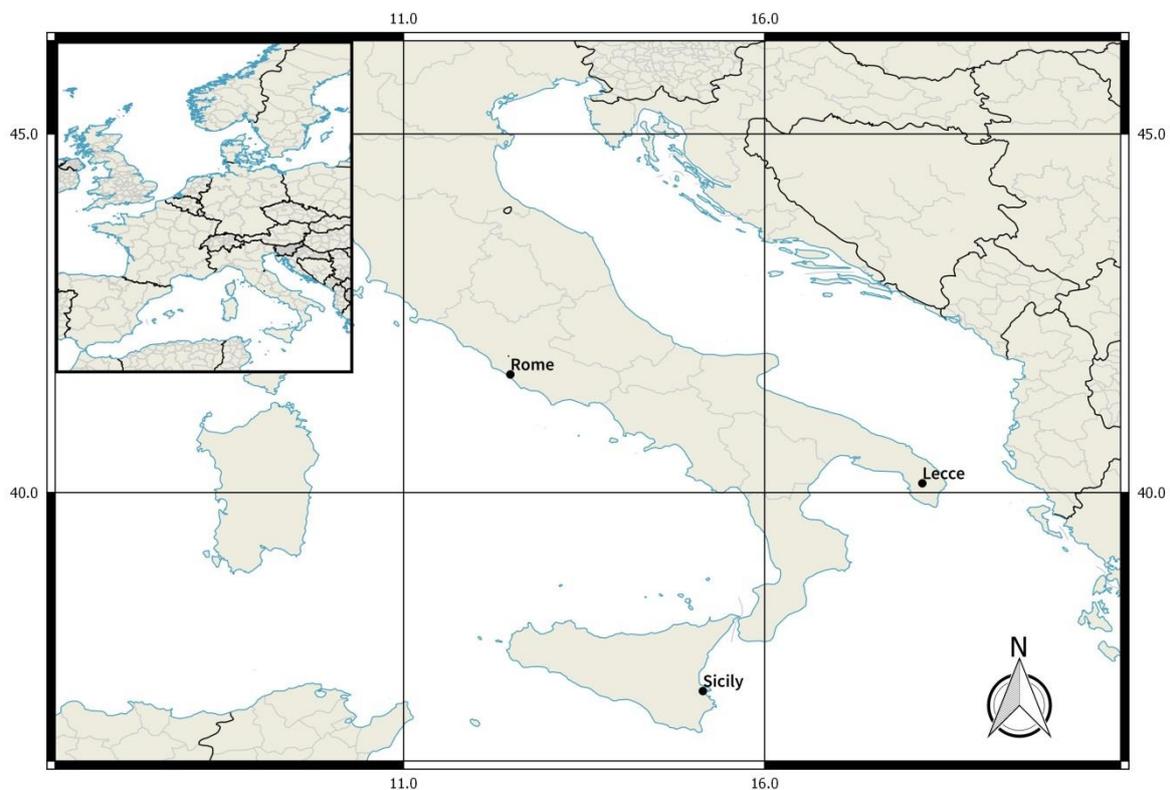
Figure 1: Map of Italy: Rome, Lecce and Sicily are the outcrops where shell samples were collected. This map was created using QGis, dataset Natural Earth quickstart kit (freely available at http://www.natrualearthdata.com).

Figure 2: (A), (B) and (C) show outcrop conditions and collected shells near Rome. (D), (E) and (F) show shells which were collected in Controfiano Park, Lecce. (D) and (F) show *A. islandica* shells and (E) *Glossus humanus*. Super glue (A), hammer (B and F), pick (D) and the brush (E) present scale bars.

Shells (52) from Sicily (July 17th, 2015; Long 37.23°, Lat 15.14°) were the best preserved ones which could be collected in all three outcrops. *A. islandica* shells were completely preserved and sometimes even both valves could be found. This outcrop was recently exposed while excavating a drainage channel of a nearby quarry. The shells were embedded in silty and sandy sediment (Fig. 3). The layer, which contains *A. islandica* shells was around 1 m below the surface (Fig. 3A).

According to preliminary biostratigraphic data all three deposits belong to the Calabrian, Middle Pleistocene, between 1.2 and 0.9 Ma for the Sicily outcrop and 1.2 and 1.4 Ma for Rome and Lecce outcrops, respectively (pers. comm. Daniele Scarponi).

Figure 3: Sicily outcrop (Long 37.23°, Lat 15.14°). (A) Trench where *A. islandica* where collected. This outcrop is characterized by silty and sandy sediment. (B) to (E) show preservation state of shells. Hammer (B), thumb (C) and chisel (D and E) represent scale bars.

After arrival all collected shells were cleaned in the lab with tap water using a toothbrush and ultrasonic bath and dried in a drying cabinet at 40°C. Some broken shells were glued together using super glue (Pattex Sekundenkleber Ultra Gel). These shells were excluded from isotopic geochemical analyses. Following, every sample was labelled and *A. islandica* shell dimensions were measured (length, height, width), weighted and photographed with a scale bar.

2.2 Identification of mollusc species

Shells of all collected molluscs and gastropods were identified using an identification key (Riedel, 1983). Furthermore, Daniele Scarponi helped identifying the associated fauna of *A. islandica*. Samples were identified to the species level when possible. When molluscan or gastropod shells were incomplete and important details were missing, they were identified to the family or genus level.

2.3 Preparation of shell cross-sections

2.3.1 Coating

In total 98 *A. islandica* shells, fragments and umbos were collected: 30 shells from Sicily and 5 shells from each Lecce and Rome were chosen for further investigations. All samples were coated twice with Araldite 2020 to prevent breaking of the shell during cutting (Fig. 4 A+B). It dried in a drying cabinet at ~40°C for at least 24 hours after every coating step before further preparation steps were performed.

2.3.2 Cutting

In order to prepare 3 mm-cross sections, the shells were cut along the line of strongest growth (LSG), which is perpendicular to the shell growth lines. Therefore, 3 mm cross-sections were prepared by cutting the embedded shells two times. Firstly, 3 mm to the right of the LSG and secondly, directly along the LSG. Umbos and smaller shells were cut using BUEHLER IsoMet Low Speed Saw. Bigger and thicker shells were cut using BUEHLER IsoMet 1000 Precision Saw (Fig. 4 C+D). Both saws were equipped with a 15LC diamond saw blade. Subsequently, cut cross-sections were mounted on glass-slides using Araldite 2020. A second cross-section of 5 mm was prepared for the eleven shells, which were chosen to be suitable for stable oxygen isotope ($\delta^{18}O_{shell}$) measurements (see Section 2.5). Those cross-sections needed to be slightly thicker (than the ones for age determination and increment width measurements) to ensure that enough carbonate powder for stable oxygen isotope ($\delta^{18}O_{shell}$) analysis can be conducted by micro-milling (c.f. Section 2.5.1).

2.3.3 Grinding

In order to gain an even surface all prepared cross-sections were ground with sandpapers of four different grain sizes (17.5 µm (P1000), 15 µm (P1200), 10 µm (P2500) and 5 µm (P4000)) using a BUEHLER Alpha 2 Speed Grinder-Polisher (low-speed) (Fig. 4 E+F). To remove residual Araldite 2020 some of the cross-sections were ground manually with sandpaper of a grain size of 25 µm (P600). Cross-sections were not polished to prevent contamination of the shells with polishing paste, which may impact stable isotope analysis.

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Figure 4: Preparation of cross-sections. (A)+(B) Coating with Araldite 2020 to prevent breaking of the shells during the preparation process. (C)+(D) Cutting the shells along the line of strongest growth (LSG). (E)+(F) Grinding of the cross-sections to obtain an even surface.

2.4   Preservation of shell material: confocal Raman microscopy

Stable oxygen isotopes are a powerful tool to reconstruct palaeo-water temperatures and palaeo-environment (e.g. Schöne et al., 2013). However, fossil shell carbonate may have altered due to diagenetic processes (Brand et al., 2011). Since water temperature reconstructions in *A. islandica* are based on stable oxygen isotope ($\delta^{18}O_{shell}$) analysis of aragonite this possibility has to be excluded (see formula (1) in section 2.5.3). Confocal Raman microscopy (CRM) is a non-destructive method to identify mineral phases and potential taphonomic alterations (e.g. recrystallization from aragonite to calcite due to high temperatures or high pressure; e.g. Maliva, 1998). This step was conducted prior to any biogeochemical analysis (i.e. stable oxygen isotopes ($\delta^{18}O_{shell}$)). Three randomly chosen measurement spots per ontogenetic year within each specimens that was chosen for stable isotope analysis were analysed to confirm that the shells still consist of aragonite.

Single spot measurements have been performed on a WITec alpha 300 R Raman microscope (using a diode laser with an extinction wavelength of 488 nm; technical settings: grating G2: 1800 g/mm, BLZ=500 nm; centre wavelength: 506.027 nm and spectral centre: 730.000 rel. 1/cm) using WITec Control software (version 4.0) to detect potential diagenetic alterations (e.g. from aragonite to calcite) by characterizing and identifying minerals (Nehrke and Nouet, 2011; Wall and Nehrke, 2012). Beforehand in-house standards for calcite and aragonite were measured to compare sample spectra to them.

2.5   Stable carbon ($\delta^{13}C_{shell}$) and oxygen ($\delta^{18}O_{shell}$) isotope analysis

2.5.1   Micro-milling

In total eleven shells were chosen for stable oxygen isotope ($\delta^{18}O_{shell}$) analysis with a main focus on shells from Sicily (nine shells) compared to one shell from Rome and Lecce, respectively. Prepared 5 mm cross-sections were used for the extraction of carbonate samples for oxygen isotope analyses by micro-milling (Dettman and Lohmann, 1995). Following the shape of the annual growth lines (c.f. Fig. 5) carbonate powder was milled from the outer shell layer in three identical consecutive years (years 9, 10 and 11) to ensure comparability between individuals. A 700 µm mill bit (Komet/Gebr. Brassler GmbH & Co. KG) mounted onto an industrial high precision drill (Minimo C121; Minitor Co., Ltd) attached on a binocular microscope (6x magnification) was used for milling (Fig. 5). Beforehand Araldite was removed on the outer shell layer. Micro-milling ensures that the samples represent the entire ontogenetic years including minimum and maximum values consequently showing seasonal variation. Calcium carbonate samples were put in crucibles and stored in aluminium trays before they were weighted and measured.
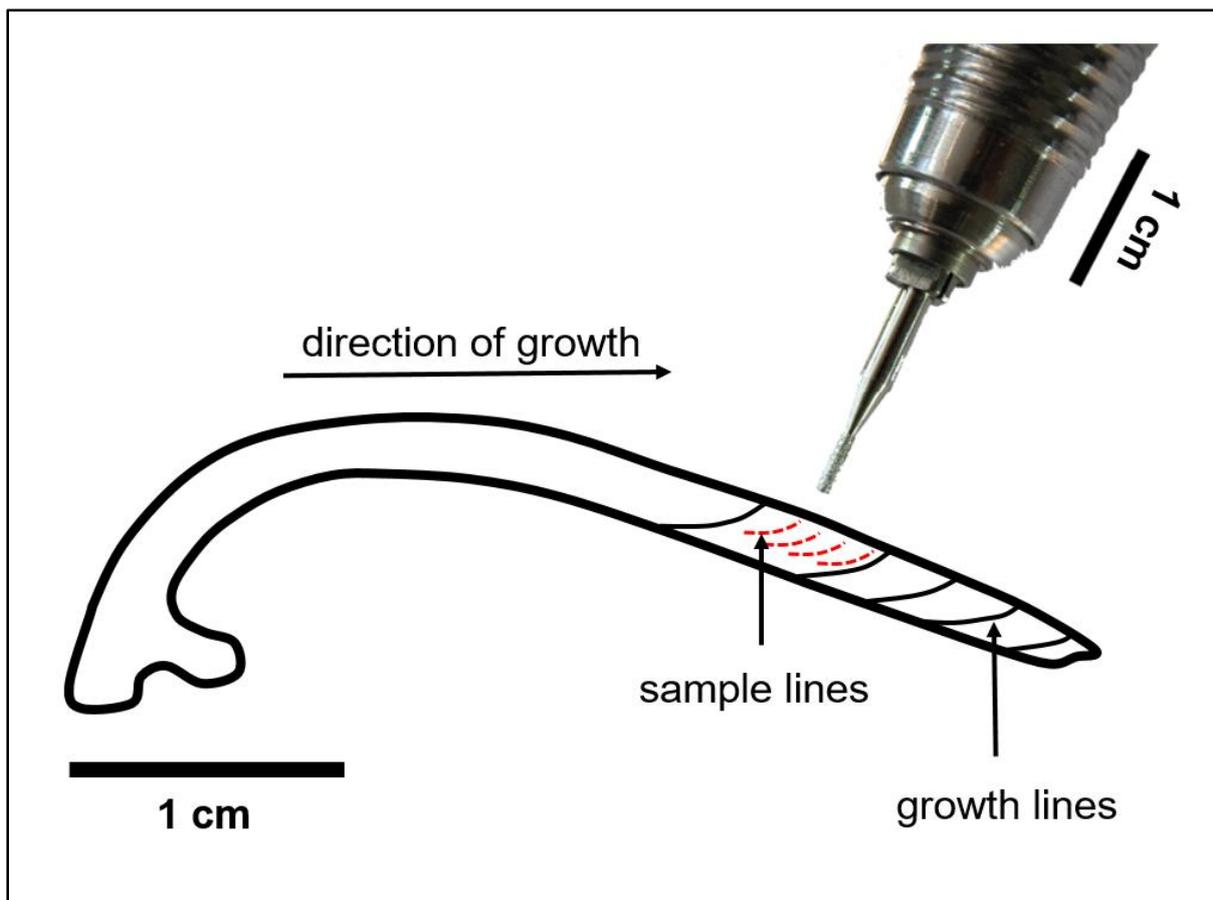
Figure 5: Micro-milling for $\delta^{18}O_{shell}$ analysis. Calcium carbonate ($CaCO_3$) samples were milled from the outer shell layer in three consecutive years using a 700 µm mill bit.

2.5.2 Isotope ratio mass spectrometry

To ensure that all isotope samples meet the weight requirement of 60 and 120 µg (with an absolute minimum of 40 µg) the samples were previously weighed with a high-resolution (µg scale) balance at the Alfred Wegner Institute, Bremerhaven, Germany. Afterwards samples were measured at the Institute of Geosciences, University of Mainz, Germany. Carbonate powder samples were dissolved with concentrated phosphoric acid in He-flushed borosilicate exetainers at 72°C and measured with a Thermo Finnigan MAT 253 continuous-flow isotope ratio mass spectrometer (IRMS) equipped with a GasBench II (Fig. 6). Data were calibrated against a NBS-19 calibrated IVA Carrara marble ($\delta^{18}O=-1.91‰$). On average, internal precision (1σ) and accuracy were better than 0.04‰ for $\delta^{18}O_{shell}$ and 0.03‰ for $\delta^{13}C_{shell}$, respectively. Stable carbon ($\delta^{13}C_{shell}$) and stable oxygen ($\delta^{18}O_{shell}$) isotopes were measured by IRMS but this study focuses only on $\delta^{18}O_{shell}$ values. Obtained stable oxygen isotope data ($\delta^{18}O_{shell}$) were checked whether the intensity is less than the interpretable range or exceeds the interpretable maximum. In this case $\delta^{18}O_{shell}$ values were excluded from further analyses.

Figure 6: Isotope ratio mass spectrometry (IRMS). (A) Aluminium tray with crucibles and samples. (B) Transferring calcium carbonate sample from crucible to borosilicate exetainers. (C) Exetainers closed with special caps, which enable measurement without reopening. (D) Thermo Finnigan MAT 253 continuous-flow isotope ratio mass spectrometer equipped with a GasBench II at the Institute of Geosciences, University of Mainz, Germany.

2.5.3 Reconstruction of palaeo-water temperatures

Water temperatures are reconstructed using Grossman and Ku (1986) equation, slightly modified by Dettman et al. (1999):

$$T_{\delta 18O} (°C) = 20.60 - 4.34 \times [\delta^{18}O_{aragonite} - (\delta^{18}O_{water} - 0.27)] \qquad (1)$$

where $\delta^{18}O_{aragonite}$ is measured relative to the Vienna PDB scale and $\delta^{18}O_{water}$ relative to the V-SMOV scale.

$\delta^{18}O$ values for seawater of the middle Calabrian Mediterranean Sea have to be estimated based on certain assumptions. According to Crippa et al. (2016) a $\delta^{18}O_{water}$ value of 0.0‰ can be assumed for shells from Pleistocene interglacials. Following Schrag et al. (2002) and Crippa et al. (2016) a $\delta^{18}O_{water}$ value of 0.8-1.0‰ is considered for shells from Pleistocene glacials and 0.5‰ for the transition between two isotope stages, respectively. Those assumptions are based on higher evaporation rates during the Pleistocene than nowadays, more humid conditions and increased fluvial runoff (Fusco, 2007).

2.6 Age determination and multi-year signals

Studies of growth time-series and ontogenetic age determination were conducted using the 3 mm cross-sections (Fig. 7), which were prepared following the instructions in Section 2.3. Annual shell growth increments were measured in umbos or along the shell towards the ventral margins using digital images. Images were taken using an Olympus DP 72 camera mounted on an Olympus SZX12 stereomicroscope. Measurements were undertaken using the software Analysis Docu 5.0. However, in some umbos and shells growth lines or patterns were rarely or not at all visible and therefore could not be considered for ontogenetic age determination or multi-year analysis.

Measured annual shell growth increments were used to compute dimensionless standard growth indices (SGI) by the means of a cubic spline (calculated using statistical software JMP version 9.0.1; SAS Institute Inc., 2007) and growth index (GI).

$$GI = \text{increment width / spline} \qquad (2)$$

$$SGI = ((GI - \text{average}) / \text{standard deviation (GI)}) \qquad (3)$$

The SGI was used to identify sub-decadal and multi-year variability in climate signals. Singular Spectrum Analysis (SSA) and Multi-Taper Method (MTM) (kSpectra; version 3.5) were used to identify multi-year signals. Procedure followed method described in Ivany et al. (2011). Window size was selected to be between one third and one half of the time series length. Number of components was chosen to be 10.
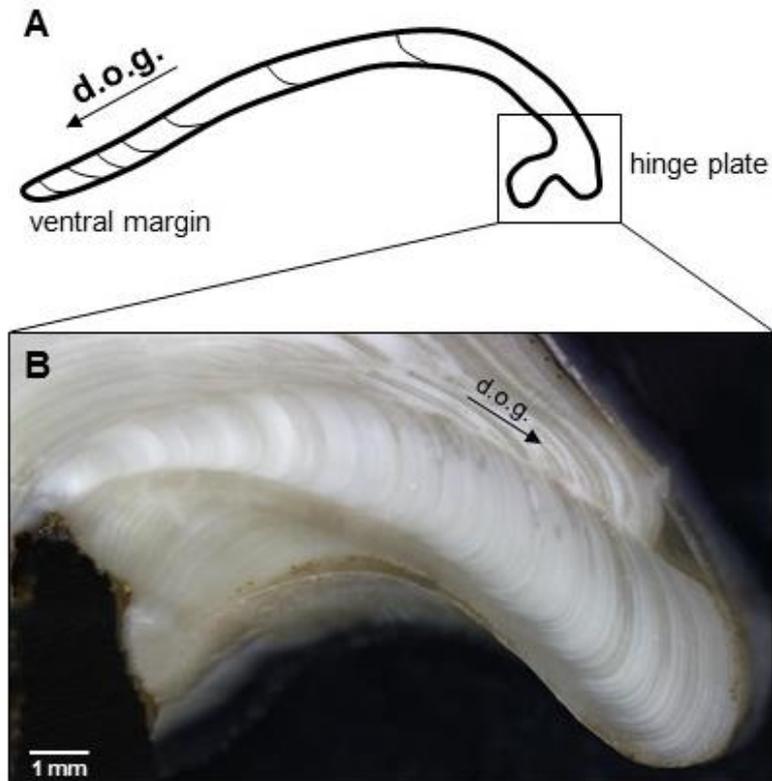
Figure 7: (A) Shell cross-section indicating area of hinge-plate. (B) Hinge plate with annual growth increments which were measured (specimen-ID VL-Siz-20). d.o.g. shows direction of growth. Images stitched with Microsoft Research Image Composite Editor.

3   Results

3.1   Associated fauna

Tab. 1 gives a first idea of the associated fauna to *A. islandica* during the Middle Pleistocene. Most of the listed species still occur in the Mediterranean Sea today while *A. islandica* is seen a "northern guest" (Raffi, 1986) and got extinct more than 9,800 years ago (Dahlgren et al., 2000). Furthermore, Tab. 1 and Fig. 8 show associated species of *A. islandica* that were collected during fieldwork in July 2015. However, it should be noted that this collection represents a biased representation of the outcrops as the main focus on the collection of *A. islandica* shells.

Table 1: Associated fauna of *A. islandica* in the Mediterranean Sea during the middle Calabrian, Pleistocene. X indicates that this species was found in the particular outcrop. Some shells could not be identified to the species level.

	Rome	Lecce	Sicily
Bivalvia			
Acanthocardia echinata muscronata	X	X	
Aequipecten opercularis	X	X	X
Anadara diluvii	X		
Chama sp.	X		
Glossus humanus	X	X	
Glycymeris insubrica	X	X	
Glycymeris sp.			X
Laevicardium oblongum			X
Mya truncata		X	
Ostrea edulis	X	X	
Papillicardium papillosum			
Pecten jacobaeus	X	X	X
Pseudamussium clavatum	X		
Pseudamussium septemradiatum	X		
Venus nux	X		
Gastropoda			
Aporrhais pespelecani	X		
Buccinidae (family)	X		
Nassarius prysmaticus	X		
Naticarius sp.	X		
Neptunea contraria			X
Turitella tricarinata pliorecens	X	X	
Xenophora crispa	X	X	
Corals	X		
Dentalium rectum/ secta	X		
(Scaphopoda)	X		
Ditrupa sp.		X	
Echinoidea		X	

Plate 1

12a   12b

13a   13b

2 cm

Plate 2

14

16

15a

17a

17b

19a

18

19b

15b

20a

20b

21a

21b

22a

22b

2 cm
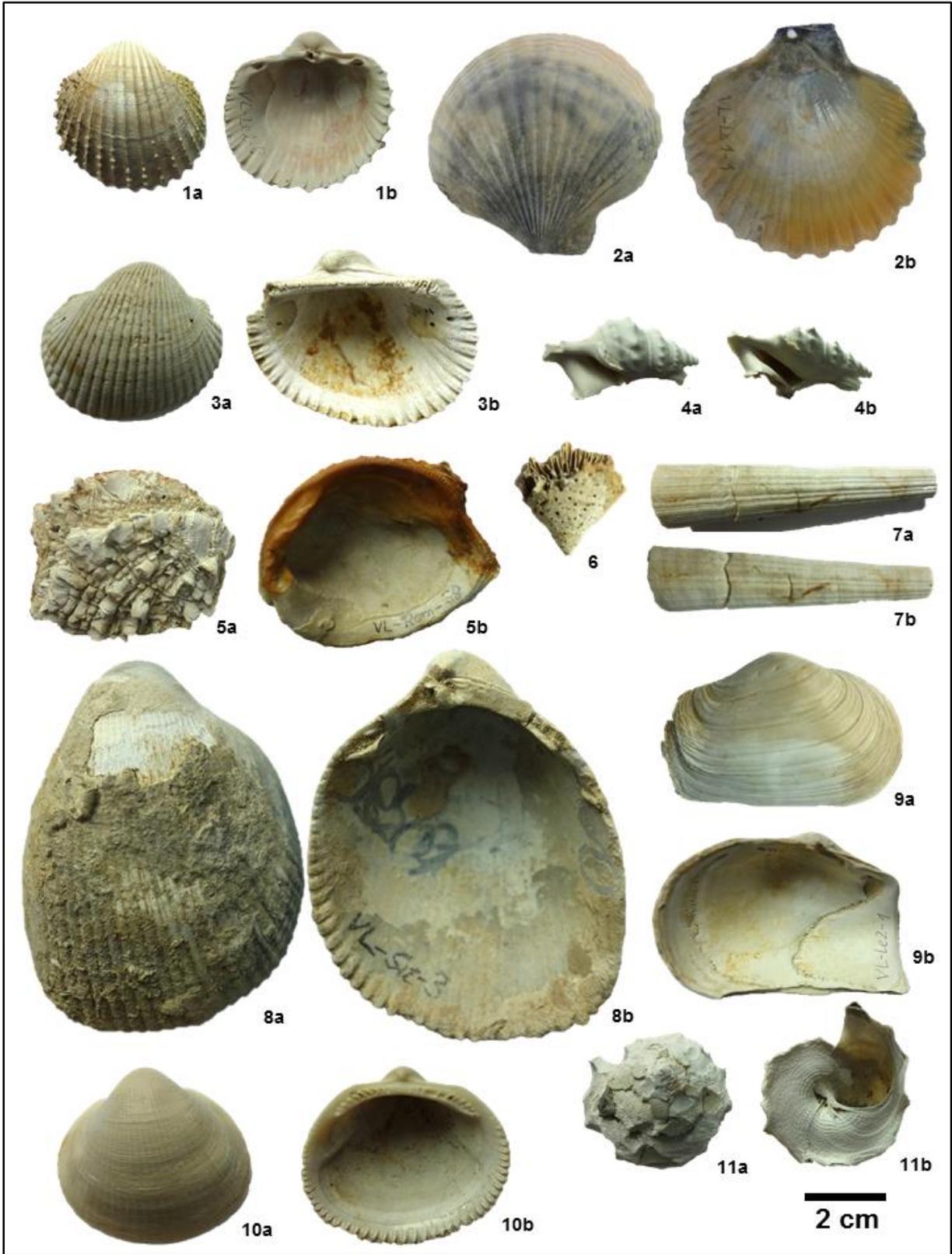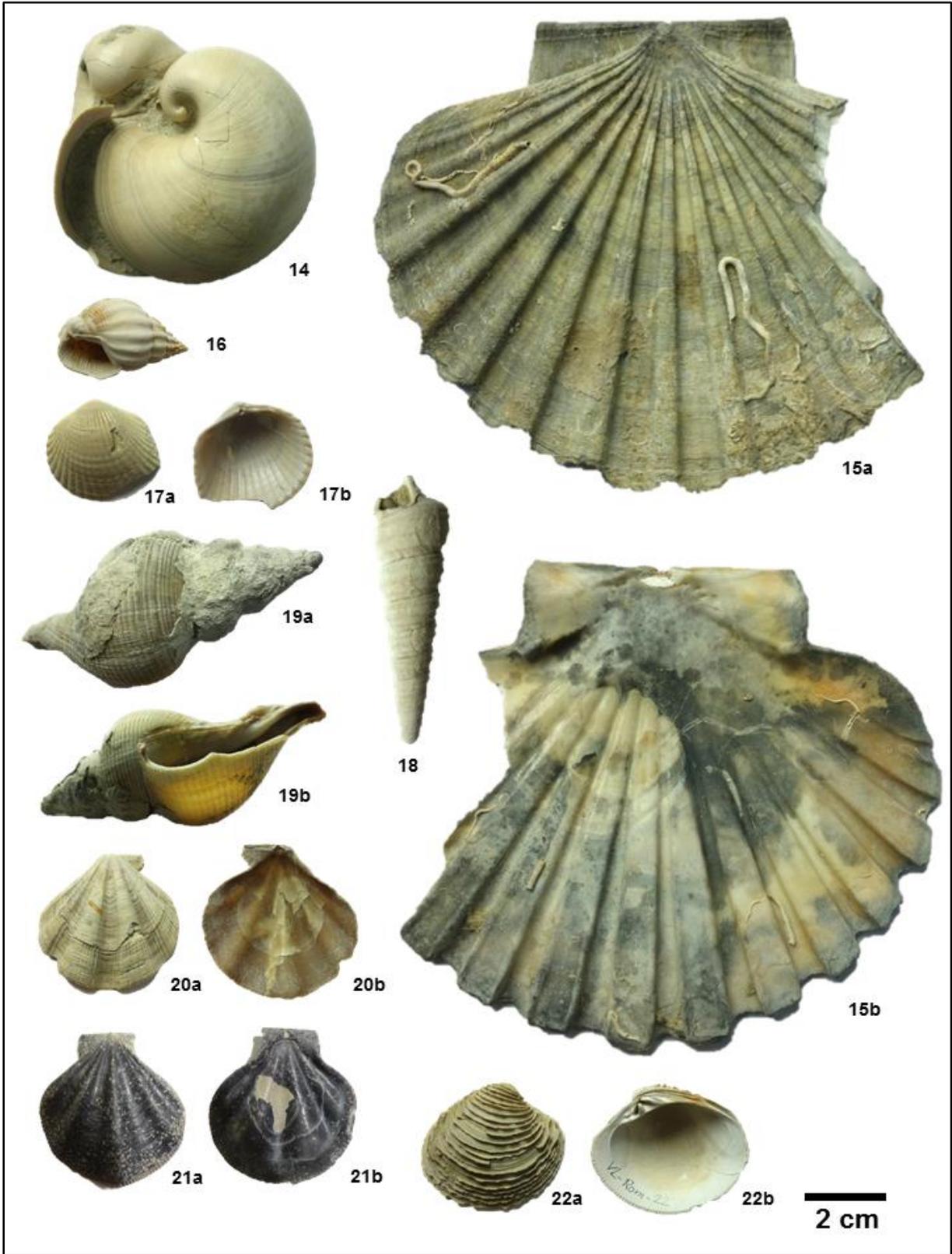
Plate 3

Figure 8: Associated fauna of *A. islandica* in the Mediterranean Sea during the Pleistocene (see Tab. 1). **Plate 1:** All specimens are shown in a) abapertural and b) apertural view except when otherwise indicated. 1a,b *Acanthocardia echinata muscronata*, 2a,b *Aequipecten opercularis*, 3a,b *Anadara dilurii*, 4a,b *Apporhais pespelecani*, 5a,b *Cham* sp., 6 Coral, 7a,b *Dentalium rectum seceta*, 8a,b *Leavicardium oblongum*, 9a,b *Mya truncata*, 10a,b *Glycymeris insubrica*, 11a,b *Xenophora crispa*. **Plate 2:** 12a,b *Ostrea edulis*, 13a,b *Glycymeris* sp. **Plate 3:** 14 *Glossus humanus*, 15a,b *Pecten jacobaeus*, 16 *Nassarius prysmaticus*, 17a,b *Papillicardium papillosum*, 18 *Turitella tricarinata pliorecens*, 19a,b *Neptunea contraria*, 20a,b *Pseudomussium clavatum*, 21a,b *Pseudomussium septemradium*, 22a,b *Venus nux*

3.2   *Preservation of shell material: confocal Raman microscopy*

The comparison of spectra from aragonite and calcite standards (both calcium carbonate polymers) to shell sample spectra is exemplarily illustrated in Fig. 9, clearly illustrating that the sample (fossil *A. islandica* shell) consists of pristine aragonite. Aragonite and sample spectra both share the characteristic double peak and do not show the calcite specific peak at ~290 nm. All spectra conducted by single spot measurements (in total 99 single spot measurements in 11 shells) do not show any signs of diagenesis. The results of the CRM analysis clearly show that all shell carbonate samples are pristine, i.e. no diagenetic alteration from aragonite to calcite occurred and thus all shells can be used for biogeochemical analysis.
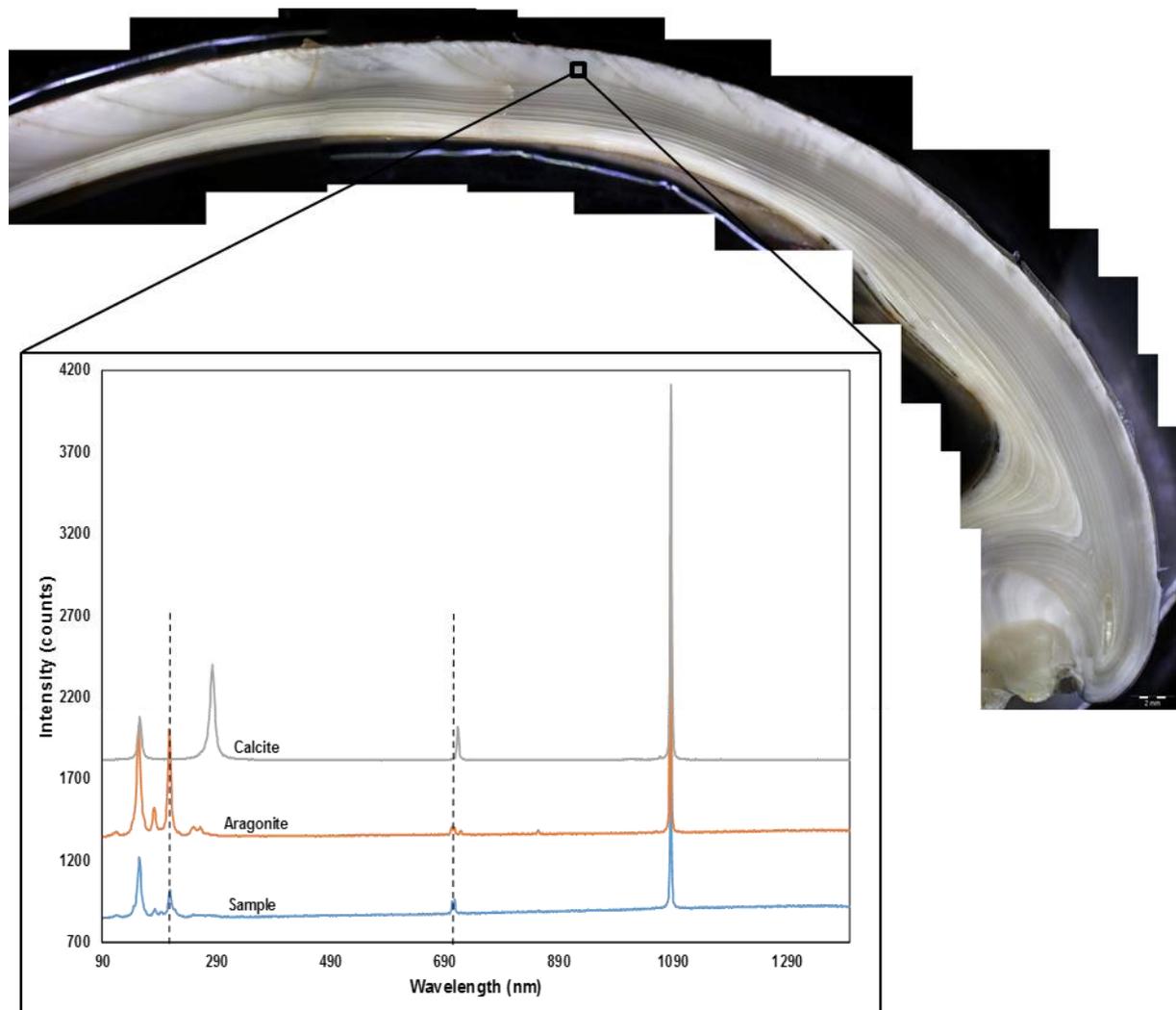
Figure 9: Confocal Raman microscopy (CRM): The black square indicates where one single spot measurement (three per ontogenetic years resulting in nine spectra per shell) was performed (specimen-ID VL-Siz-58). Scale-bar on shell cross-section is 2 mm. CRM clearly shows that the sample does not show signs of diagenesis. Sample (blue) and aragonite (orange) standard spectra show identical peaks whereas the calcite (grey) standard spectrum differs from the sample spectra.

3.3 Stable oxygen isotopes ($δ^{18}O_{shell}$)

In total 496 measurements of stable oxygen ($δ^{18}O_{shell}$) and stable carbon ($δ^{13}C_{shell}$) isotopes were conducted by IRMS. On average 45 carbonate samples were taken per shell (15 per ontogenetic year), with a minimum of 27 samples in shell VL-Siz-47 and a maximum of 63 samples in shell VL-Le3-3. The number of samples varied due to different increment widths. This study focuses on stable oxygen isotopes, but stable carbon isotopes were also measured by IRMS. Tab. 2 presents information on shell morphology, stable oxygen isotope measurements, ontogenetic ages for all fossil shell specimens considered in this study and whether the shell has been used for multi-year analysis. Detailed information on minimum, maximum and average stable oxygen isotope ($δ^{18}O_{shell}$) values as well as $δ^{18}O_{shell}$ amplitude and the number of calcium carbonate samples taken are shown in Tab. 3. All $δ^{18}O_{shell}$ profiles show low intra-annual, i.e. seasonal variability (Figs 10&11). The $δ^{18}O_{shell}$ amplitude varies between 0.4‰ (VL-Le3-3) and 1.1‰ (VL-Siz-41). Since a difference of 1.0‰ is equivalent to a temperature variation of 4.34°C those values result in a seasonal temperature variation of 1.74°C and 4.82°C, respectively. However, this seasonality range is just correct when salinity was absolutely constant over time since stable oxygen isotopes ($δ^{18}O_{shell}$) are a dual proxy for water temperatures and salinity. The average $δ^{18}O_{shell}$ amplitude is 0.67‰ indicating an average seasonal variation of 2.9°C.

Analysed isotope geochemical data reveal relatively constant $δ^{18}O_{shell}$ values over the measured time period of three years. Minimum values of $δ^{18}O_{shell}$ are close to or at the grey winter lines. $δ^{18}O_{shell}$ values increase after the grey winter lines (Fig. 10b,c). Maximum $δ^{18}O_{shell}$ values are usually observed in the first half of the white area (Fig. 10).

Table 2: Shell information. Information on shell morphology, stable oxygen isotope measurements and ontogenetic ages for all fossil shell specimens. Cross (X) indicates if stable oxygen isotopes ($\delta^{18}O$) were measured and/or multi-year analysis was conducted. A minus (-) shows that stable oxygen isotopes ($\delta^{18}O$) were not measured and/or shells were not considered for multi-year analysis, due to short length of time-series (for frequency analysis time-series longer than 50 years were considered). (*) Number of years which could be measured and were considered in frequency analysis, however shell is considered older since not all increments were visible. (+) Measurements should be repeated.

Shell-ID	Preservation	Length (mm)	Height (mm)	Width (mm)	Weight (g)	$\delta^{18}O_{shell}$ data	Ontogenetic age (yr)	Multi-year signals
V-Siz-14	incomplete valve	87.4	84.68	23.64	61.1	-	175	X
VL-Siz-20	fragment with umbo	-	-	-	31.0	-	47	-
VL-Siz-23	fragment with umbo	-	-	26.74	81.7	X	99	X
VL-Siz-33	incomplete valve	-	91.96	27.07	88.7	X	202	X
VL-Siz-35	complete valve	98.3	93.08	28.12	91.0	X	87	X
VL-Siz-36	complete valve	78.6	73.0	22.86	47.7	-	20 umbo 24 ventral margin	-
VL-Siz-39	complete valve	99.5	89.36	27.21	80.2	X	110	X
VL-Siz-41	complete valve	107.4	104.28	32.38	151.6	X	>300+	-
VL-Siz-46	incomplete valve	-	89.58	25.58	60.9	-	120	X
VL-Siz-47	fragment with umbo	-	-	23.01	51.8	X	-	-
VL-Siz-52	fragment with umbo	93.2	-	28.31	75.3	-	48	-
VL-Siz-55	fragment with umbo	-	-	27.77	75.3	X	98*	X
VL-Siz-56	incomplete valve	-	89.19	25.81	71.3	X	106	X
VL-Siz-58	complete valve	87.8	87.36	25.42	70.4	X	68	X
VL-Siz-60	incomplete valve	-	92.96	27.03	92.2	-	177	X
VL-Rom-57	umbo	-	-	-	25.8	-	151	X
VL-Rom-60	umbo	-	-	-	18.3	-	113	X
VL-Rom-61	fragment with umbo	-	-	25.70	83.4	X	>155+	-
VL-Le3-3	complete valve	94.6	94.64	27.47	86.8	X	132	X

Table 3: Information on stable oxygen isotopes ($\delta^{18}O_{shell}$) from eleven *A. islandica* specimens. Minimum (min), maximum (max) and average $\delta^{18}O_{shell}$ value are given. $\delta^{18}O_{shell}$ average (‰): average of all measured $\delta^{18}O_{shell}$ values in a specific shell. $\delta^{18}O_{shell}$ amplitude (‰): difference between maximum and minimum $\delta^{18}O_{shell}$ values. Number of samples taken for stable oxygen isotope ($\delta^{18}O_{shell}$) measurements. Number of samples varies depending on the width of annual growth increment. Seasonal temperature variation is calculated on the assumption that a difference of 1.0‰ is equivalent to a temperature variation of 4.34°C.

Shell-ID	$\delta^{18}O_{shell}$ min (‰)	$\delta^{18}O_{shell}$ max (‰)	$\delta^{18}O_{shell}$ average (‰)	$\delta^{18}O_{shell}$ amplitude (‰)	Seasonal temperature variation (°C)	Number of samples
VL-Siz-23	2.7	3.5	3.1	0.7	3.3	40
VL-Siz-33	2.5	3.2	2.9	0.7	3.1	40
VL-Siz-35	2.6	3.5	3.1	0.8	3.7	57
VL-Siz-39	2.8	3.3	3.1	0.6	2.4	39
VL-Siz-41	2.7	3.8	3.4	1.1	4.8	59
VL-Siz-47	1.9	2.3	2.1	0.4	1.9	27
VL-Siz-55	3.3	4.2	3.8	0.9	3.9	37
VL-Siz-56	2.9	3.4	3.2	0.5	2.0	46
VL-Siz-58	3.7	4.3	4.1	0.5	2.3	36
VL-Rom-61	3.2	3.8	3.5	0.6	2.7	52
VL-Le3-3	3.2	3.6	3.4	0.4	1.7	63

d) VL-Siz-39

e) VL-Siz-41

f) VL-Siz-47

g) VL-Siz-55

h) VL-Siz-56

i) VL-Siz-58
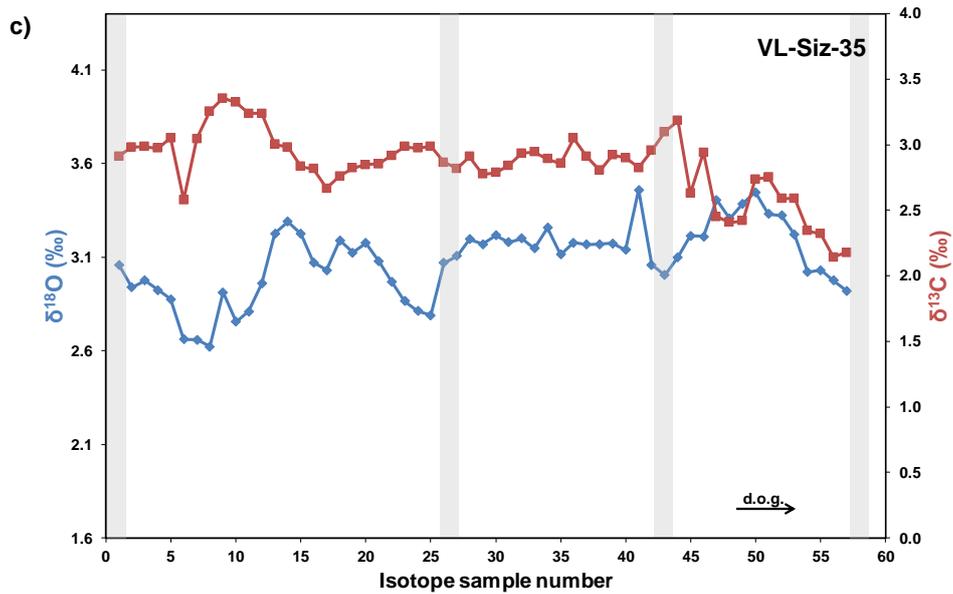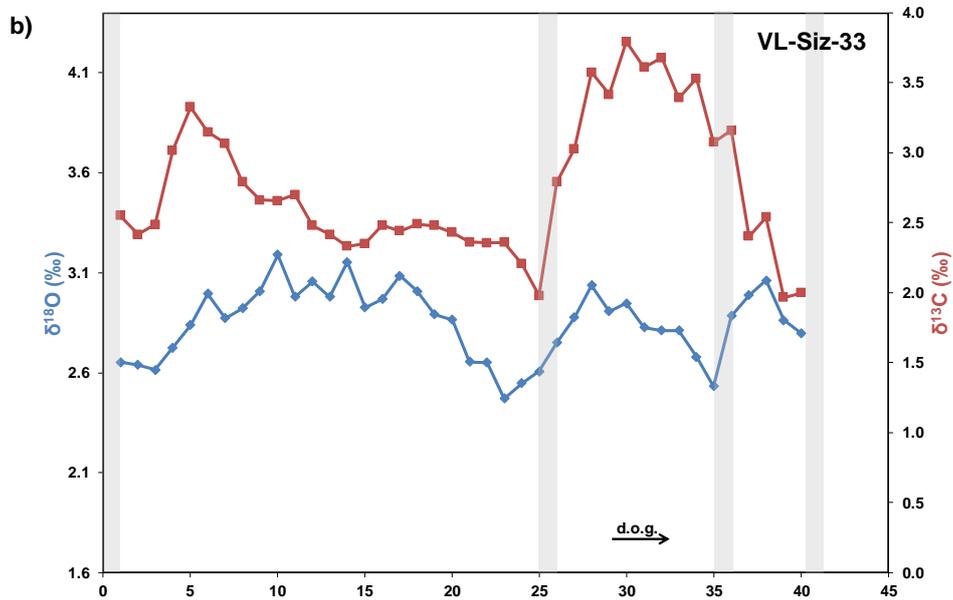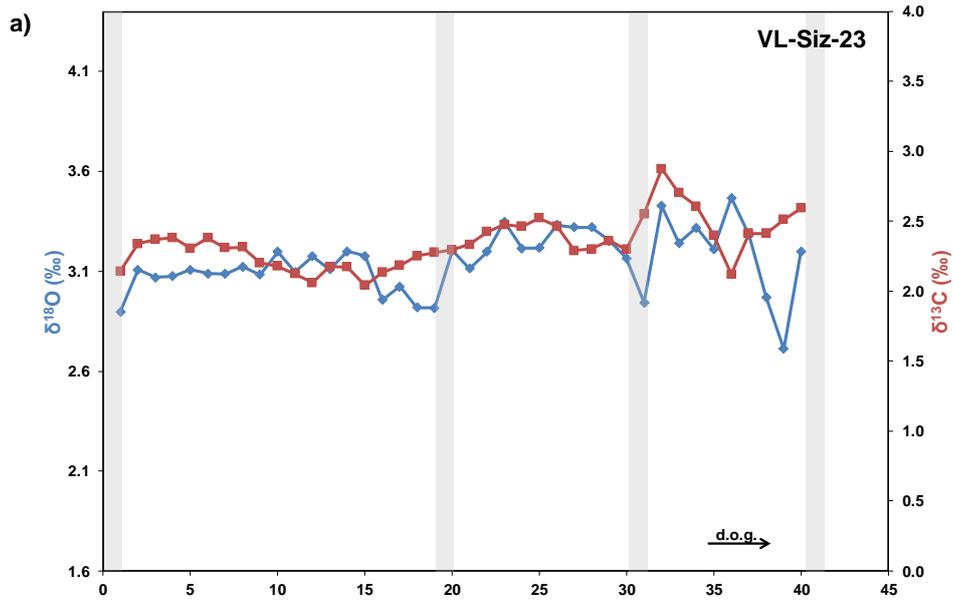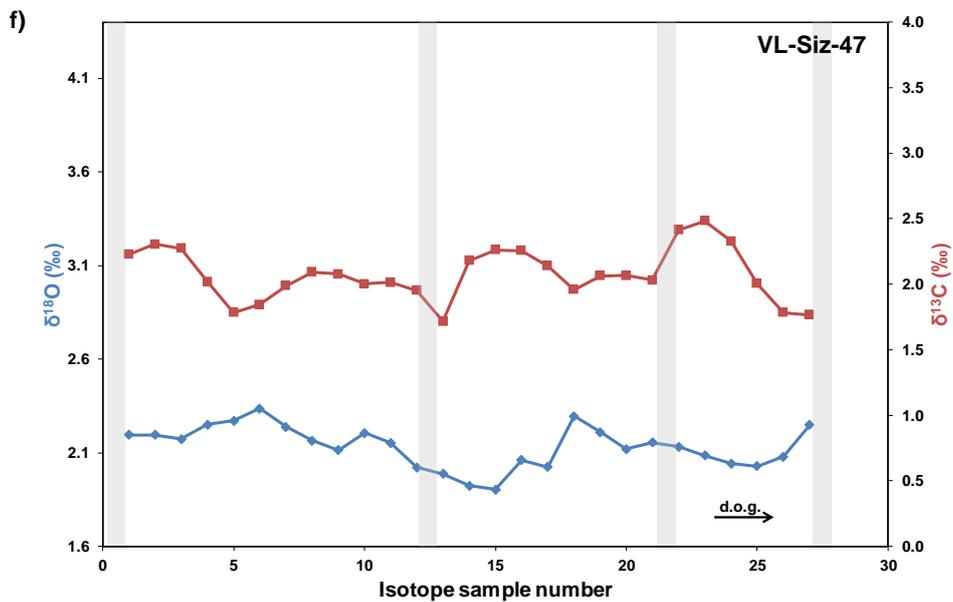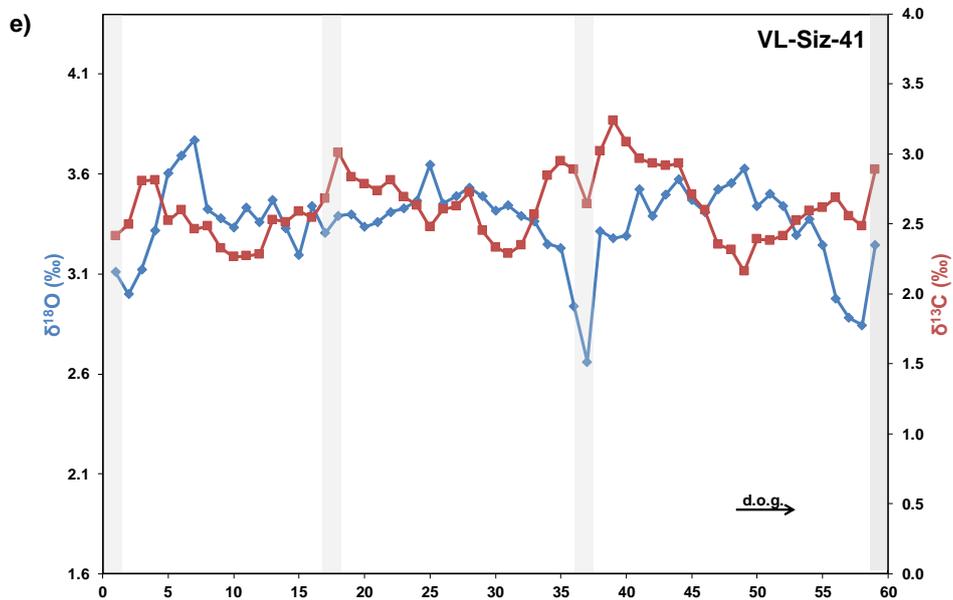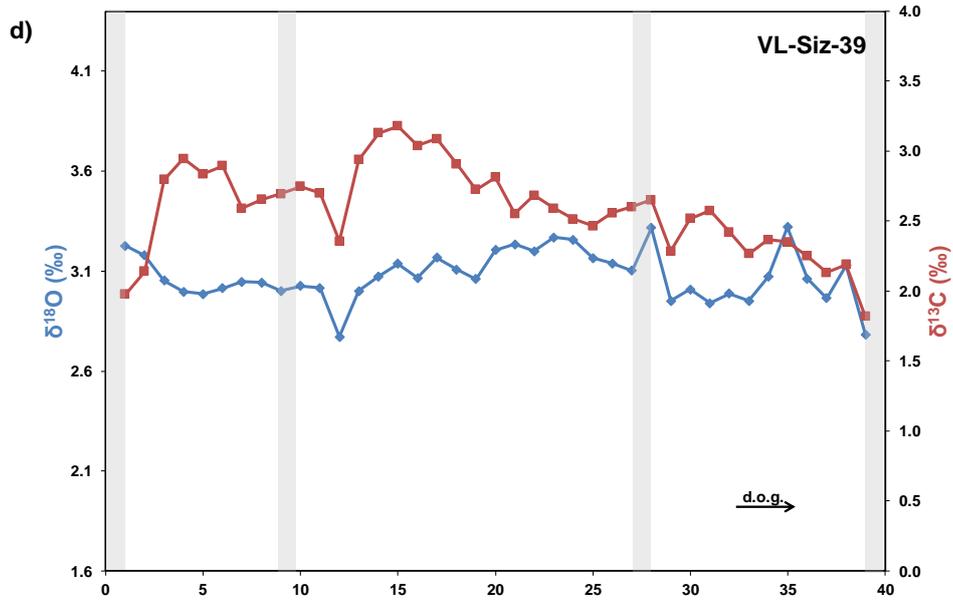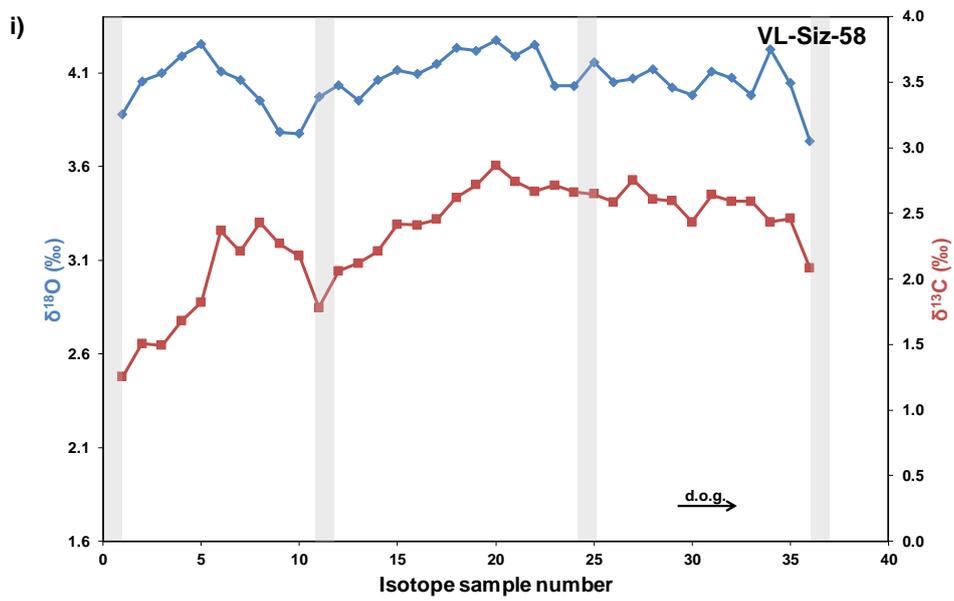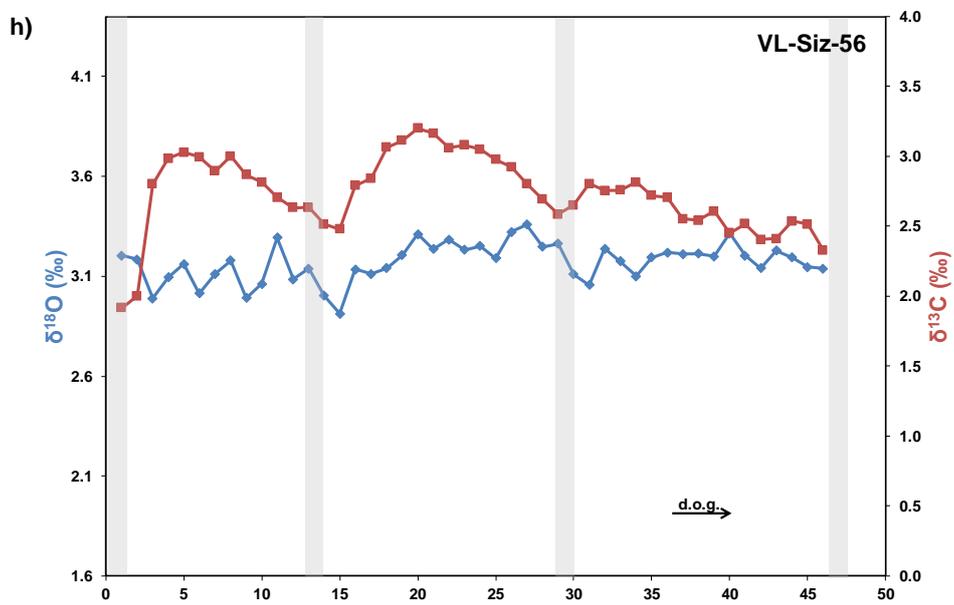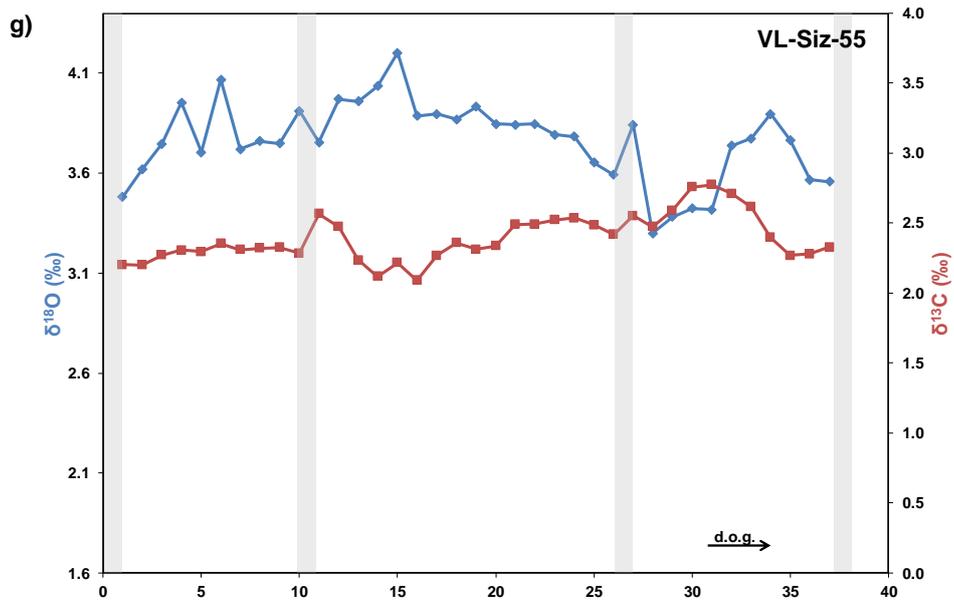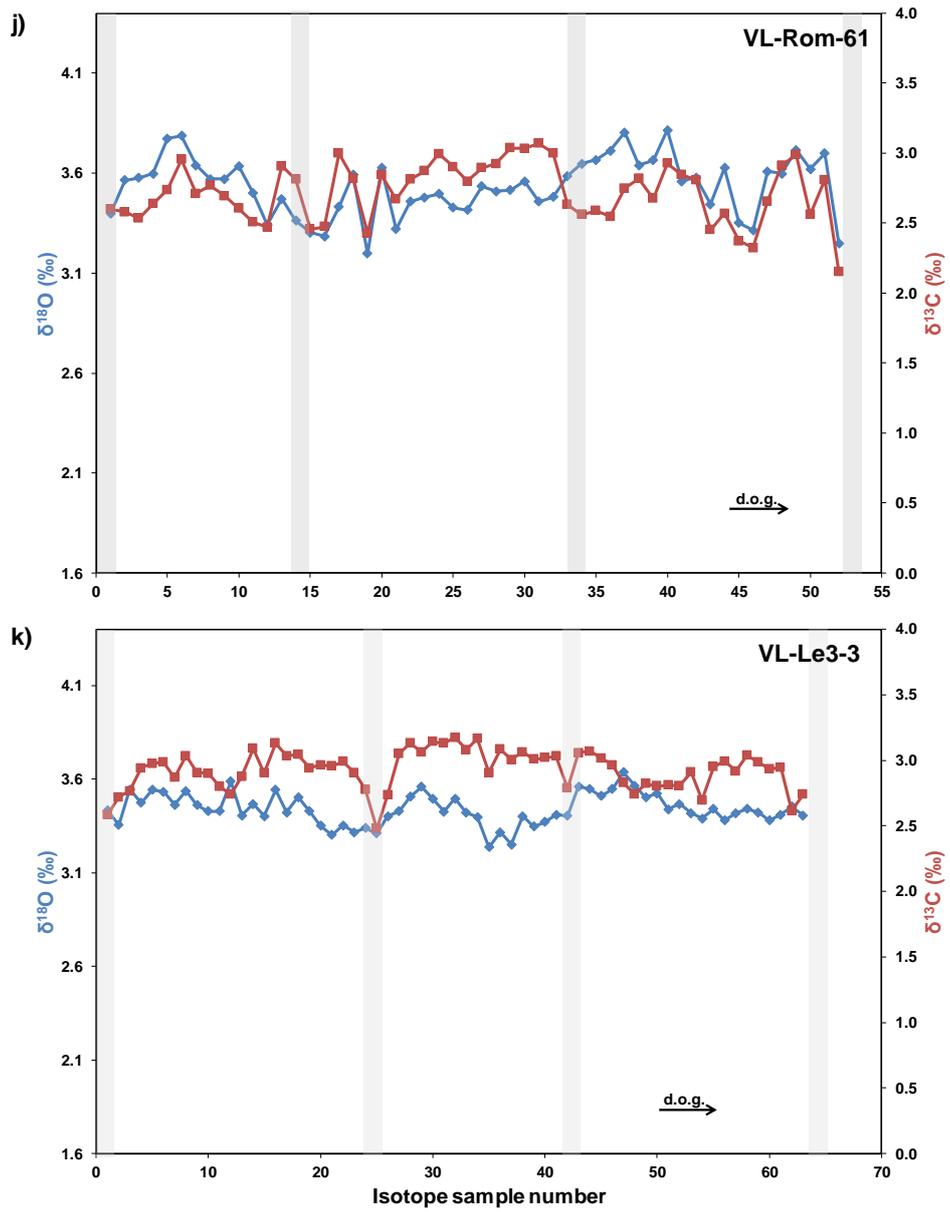
Figure 10: $\delta^{18}O_{shell}$ (blue) and $\delta^{13}O_{shell}$ (red) isotope profiles of eleven *A. islandica* shells which were measured by isotope ratio mass spectrometer. Grey bars represent growth lines. Isotope samples were taken in three consecutive ontogenetic years.

3.4 Stable carbon isotopes ($\delta^{13}C_{shell}$)

Generally, the $\delta^{13}C_{shell}$ amplitude, with the exception of three shells, is bigger than the $\delta^{18}O_{shell}$ amplitude. Shell VL-Siz-23 and VL-Siz-41 have the same $\delta^{13}C_{shell}$ and $\delta^{18}O_{shell}$ variation. Whereas, in shell VL-Siz-55 the stable oxygen isotope amplitude is larger than the stable carbon isotope amplitude (Fig.10g, Tab. 4). To check if there is a correlation between carbon ($\delta^{13}C_{shell}$) and oxygen ($\delta^{18}O_{shell}$) isotopes they were plotted against each other and the correlation coefficient R^2 was calculated. Values vary between $R^2 = 0.0057$ and $R^2 = 0.1774$ (Tab. 4, Appendix 1).

Table 4: $\delta^{13}C_{shell}$ and $\delta^{18}O_{shell}$ amplitude measured in eleven *A. islandica* shells chosen to be suitable for isotope measurements. Moreover, the correlation coefficient R^2 between stable carbon and oxygen isotopes is shown.

Specimen-ID	$\delta^{13}C_{shell}$ amplitude	$\delta^{18}O_{shell}$ amplitude	R^2
VL-Siz-23	0.8	0.8	0.0532
VL-Siz-33	1.8	0.7	0.0083
VL-Siz-35	1.2	0.8	0.1228
VL-Siz-39	1.4	0.5	0.0209
VL-Siz-41	1.1	1.1	0.0321
VL-Siz-47	0.8	0.4	0.0886
VL-Siz-55	0.7	0.9	0.1774
VL-Siz-56	1.3	0.4	0.0057
VL-Siz-58	1.6	0.5	0.0988
VL-Le3-3	0.7	0.4	0.1419
VL-Rom-61	0.9	0.6	0.0107

3.5 Palaeo-temperatures

Based on the obtained $\delta^{18}O_{shell}$ data (Tab. 3) palaeo-temperatures were reconstructed resulting in a seasonal temperature variation from 1.7°C (VL-Le3-3) to 4.8°C (VL-Siz-41) when salinity ($\delta^{18}O_{water}$) is absolutely constant and does not vary over time. Calculated minimum, maximum and average temperatures for each specimen are presented in Tab. 5. Reconstructed minimum and maximum palaeo-temperature values depend on salinity estimations due to a lack in direct $\delta^{18}O_{water}$ measurements. The high abundance of boreal guests in all outcrops in combination with the results for the stable oxygen isotope analysis ($\delta^{18}O_{shell}$) suggest assigning the outcrop to a glacial phase (pers. comm. Daniele Scarponi). Following Schrag et al. (2002) and Crippa et al. (2016) Pleistocene salinity in the Mediterranean seawater was estimated to be 0.9±0.1‰ resulting in calculated palaeo-temperatures using $\delta^{18}O_{water}$ values of 0.8‰, 0.9‰ and 1.0‰, respectively (Fig. 11).

The Sicilian specimens are characterized by an average minimum water temperature of 7.8°C (estimated $\delta^{18}O_{water}$ 0.8‰, 8.2°C for 0.9‰, 8.6°C for 1.0‰) increasing to an average maximum

temperature of 10.8°C (estimated $\delta^{18}O_{water}$ 0.8 ‰, $\delta^{18}O_{water}$ 0.9‰: 11.2°C and $\delta^{18}O_{water}$ 1.0‰: 11.7°C). The minimum water temperature recorded is 4.4°C ($\delta^{18}O_{water}$ 0.8 ‰, $\delta^{18}O_{water}$ 0.9‰: 4.8°C and $\delta^{18}O_{water}$ 1.0‰: 5.2°C in shell VL-Siz-58). Moreover, the maximum reconstructed temperature is 14.6°C ($\delta^{18}O_{water}$ 0.8‰, $\delta^{18}O_{water}$ 0.9‰: 15.1°C, $\delta^{18}O_{water}$ 1.0‰: 5.4°C in shell VL-Siz-47).

Table 5: Reconstructed water temperatures based on analysed stable oxygen isotope ($\delta^{18}O_{shell}$) data (Tab. 3) from eleven *A. islandica* specimens using different $\delta^{18}O_{water}$ values (following Schrag et al., 2002 and Crippa et al., 2016). Minimum (min), maximum (max) and average palaeo-temperatures were reconstructed using the modified Grossman and Ku (1986) equation (see Section 2.5.3).

Shell-ID	V-SMOV 0.8 ‰			V-SMOV 0.9 ‰			V-SMOV 1.0 ‰			
	T min (°C)	T max (°C)	T average (°C)	T min (°C)	T max (°C)	T average (°C)	T min (°C)	T max (°C)	T average (°C)	T amplitude (°C)
VL-Siz-23	7.9	11.1	9.2	8.3	11.6	9.7	8.7	12.0	10.1	3.3
VL-Siz-33	9.1	12.2	10.5	9.5	12.6	11.0	9.9	13.0	11.4	3.1
VL-Siz-35	7.9	11.5	9.5	8.3	12.0	9.9	8.8	12.4	10.4	3.6
VL-Siz-39	8.5	10.9	9.5	8.9	11.3	10.0	9.4	11.7	10.4	2.4
VL-Siz-41	6.5	11.4	8.3	7.0	11.8	8.8	7.4	12.2	9.2	4.8
VL-Siz-47	12.8	14.6	13.6	13.2	15.1	14.1	13.6	15.5	14.5	1.9
VL-Siz-55	4.7	8.6	6.6	5.1	9.0	7.0	5.5	9.4	7.4	3.9
VL-Siz-56	8.3	10.3	9.1	8.8	10.7	9.6	9.2	11.1	10.0	1.9
VL-Siz-58	4.4	6.7	5.2	4.8	7.1	5.7	5.2	7.6	6.1	2.3
VL-Rom-61	6.3	9.0	7.6	6.8	9.5	8.0	7.2	9.9	8.4	2.7
VL-Le3-3	7.1	8.9	8.0	7.6	9.3	8.4	8.0	9.7	8.8	1.7

d) VL-Siz-39

e) VL-Siz-41

f) VL-Siz-47

Isotope sample number
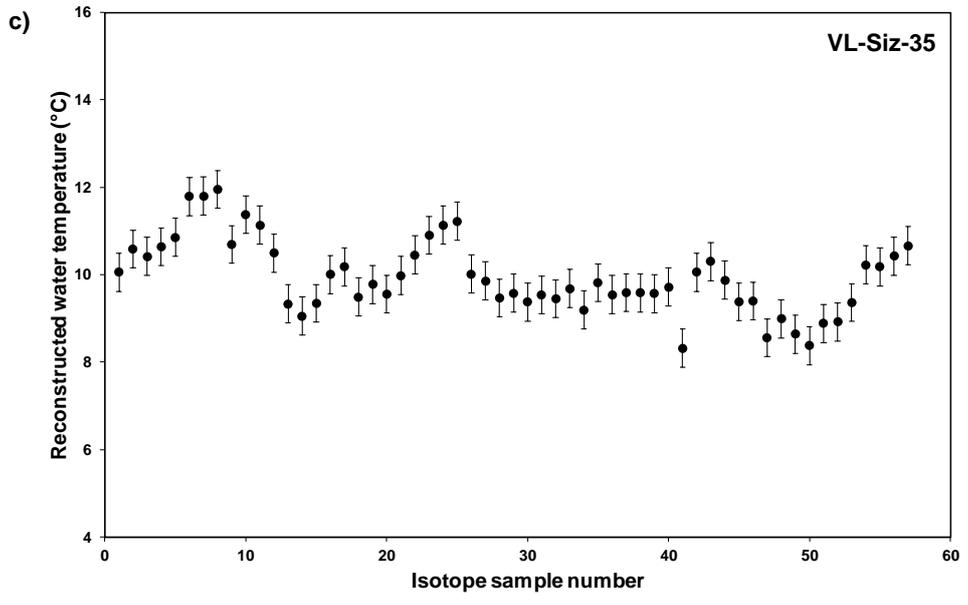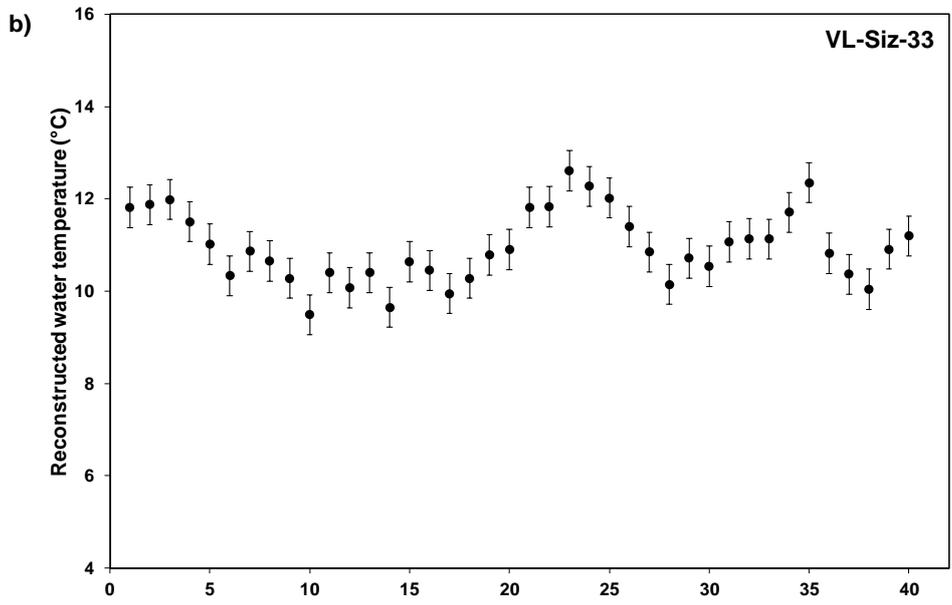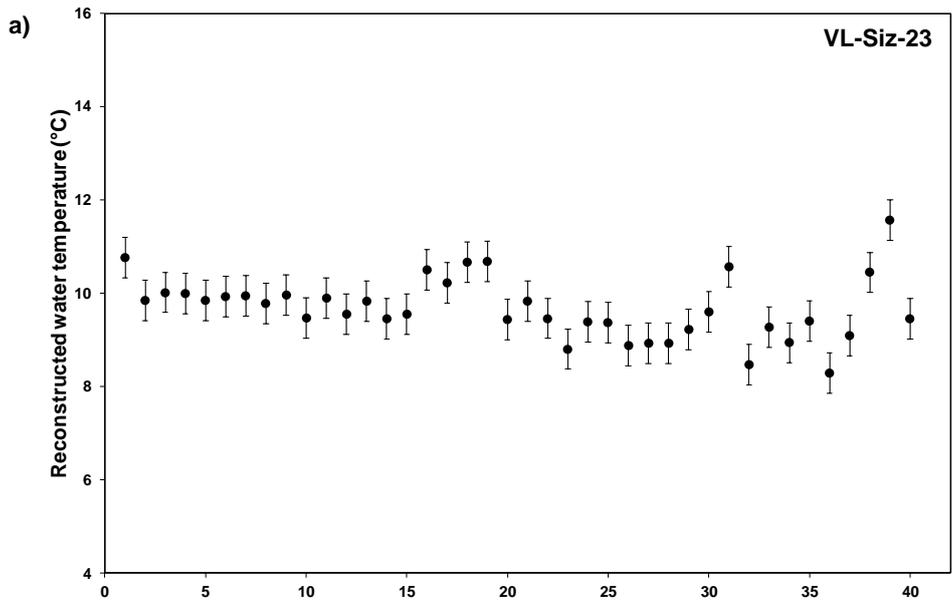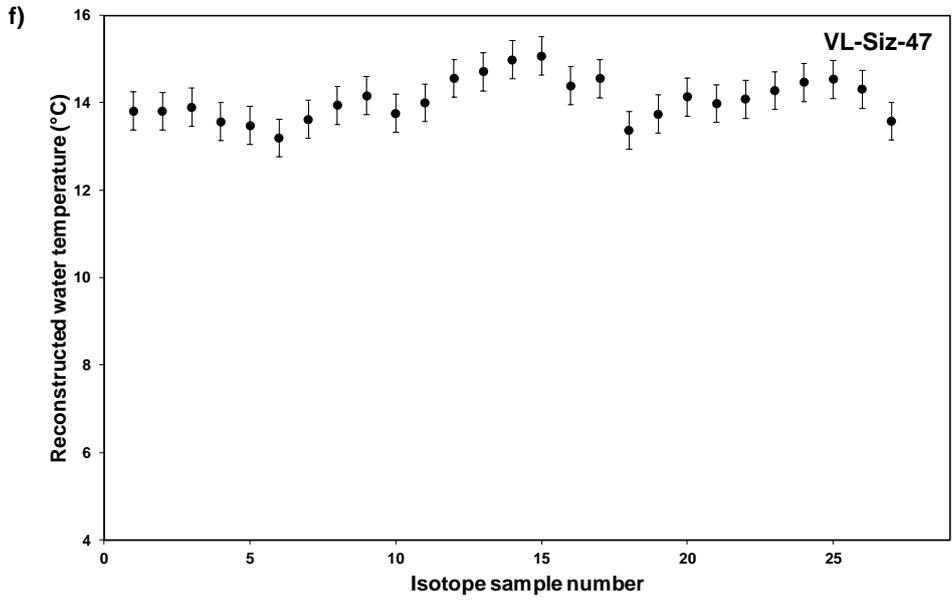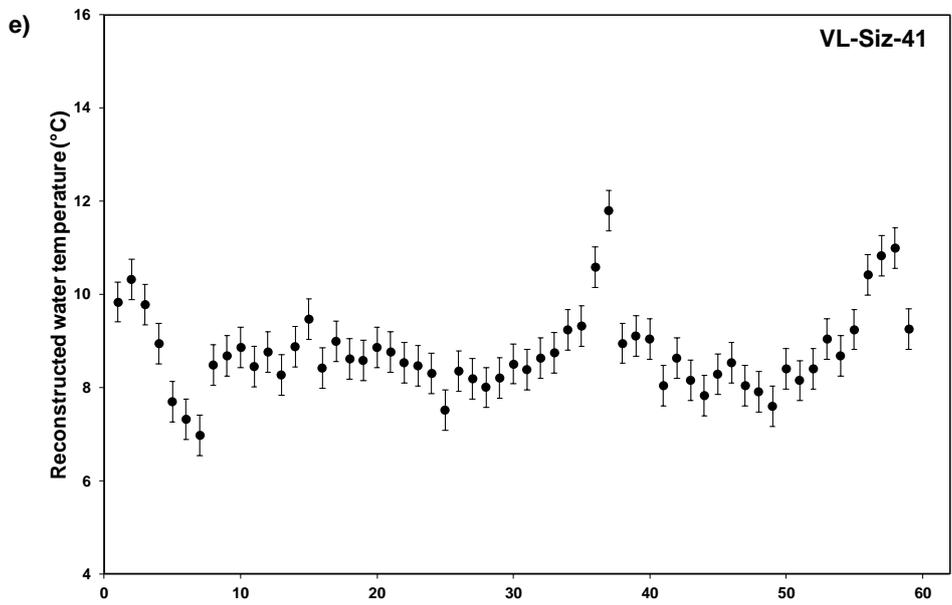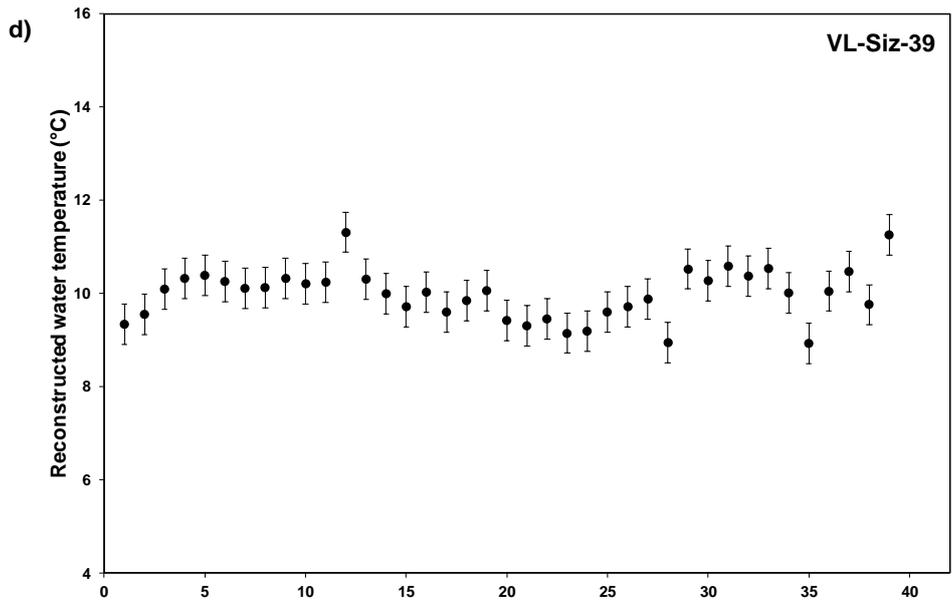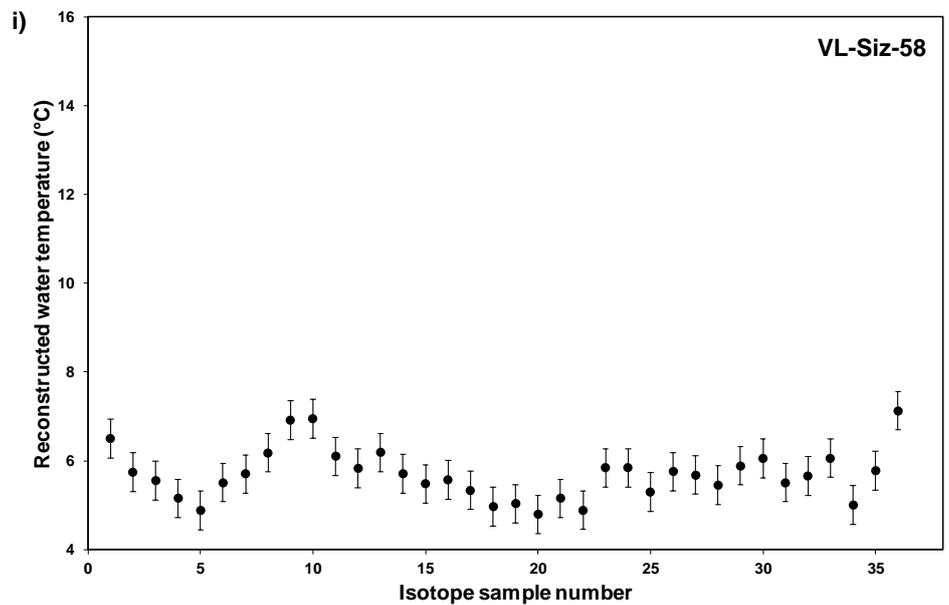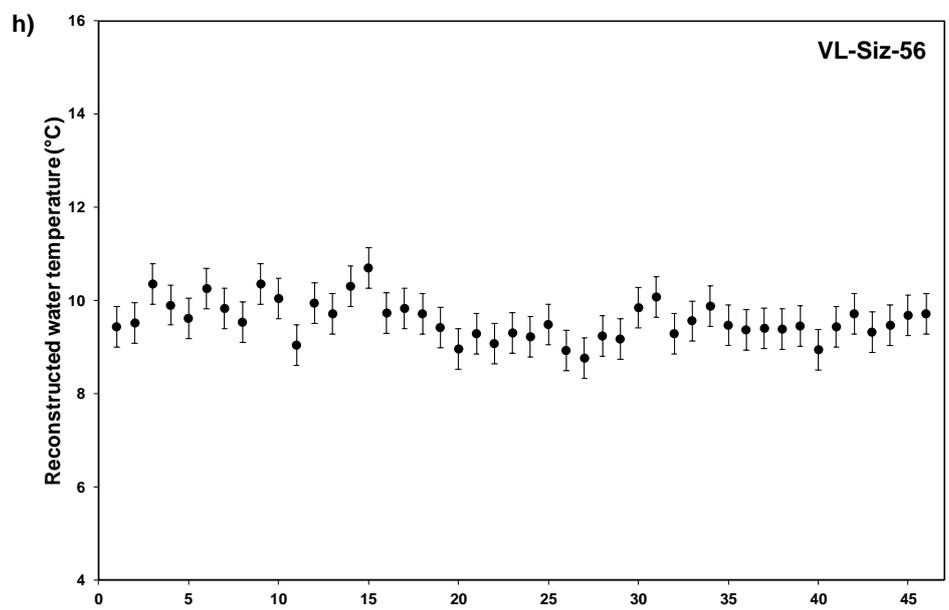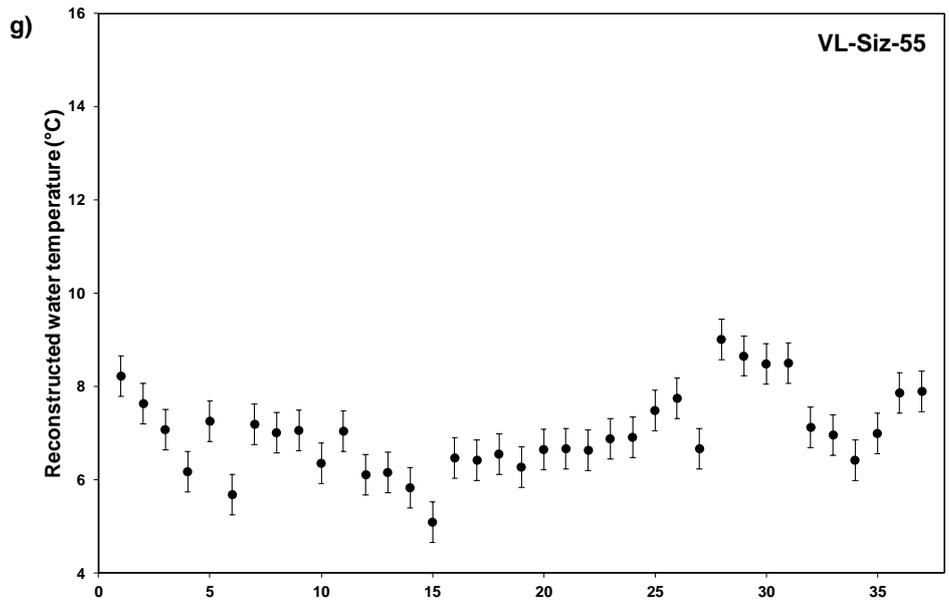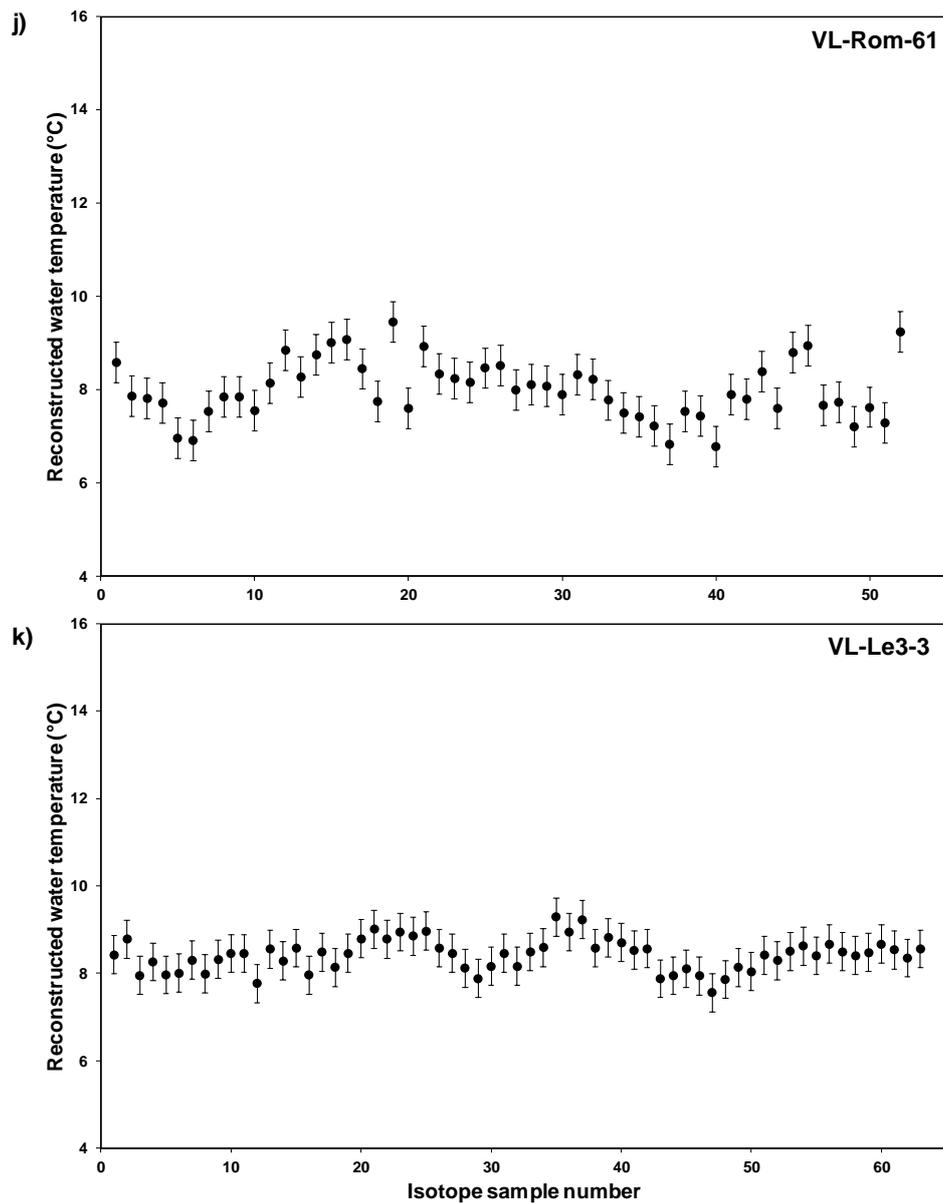
Figure 11: Reconstructed water temperatures and seasonality. Temperatures derived from stable oxygen isotope values ($\delta^{18}O_{shell}$) from eleven fossil *A. islandica* shells. $\delta^{18}O_{water}$ values of 0.9±0.1‰ have been assumed for temperature reconstructions and are given as error bars (0.8‰ and 1.0‰ respectively). (a)-(i) Shells were collected on Sicily: a) VL-Siz-23, b) VL-Siz-33, c) VL-Siz-35, d) VL-Siz-39, e) VL-Siz-41, f) VL-Siz-47, g) VL-Siz-55, h) VL-Siz-56, i) VL-Siz-58 and j) from Rome: VL-Rom-61, k) from Lecce: VL-Le3-3.

3.6   *Multi-year signals in the Pleistocene Mediterranean Sea*

By means of Singular Spectrum Analysis (SSA) and Multi-Taper Method (MTM) a prominent frequency of ~0.16 1/yr was identified in most shells (Appendix 2). This frequency is equal to a multi-year variability of ~6 years (range 5-7 years). Fig. 12 exemplarily shows the results for frequency analysis (specimen-ID: VL-Siz-23). The prominent 6-year signal has a significance of 99% in both, the original (Fig. 12c) and the reconstructed, i.e. random noise components are left behind (Fig. 12d), time-series and is also well visible in Fig. 12b, representing the results of SSA. If the black dots are above the red noise range (red bars), the frequency is significant in terms of SSA.
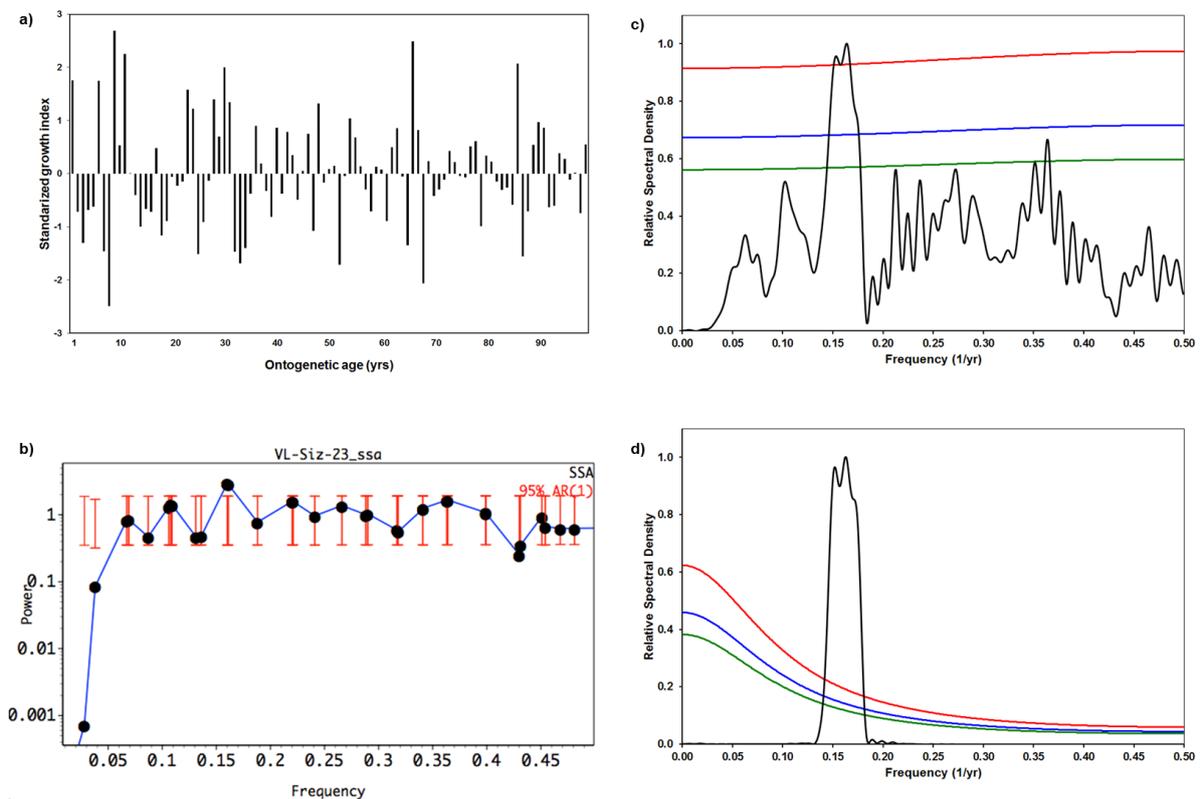
Figure 12: Frequency analysis – exemplarily presentation for shell VL-Siz-23. (a) Standardized growth index, (b) Singular-Spectrum Analysis and (c and d) Multi-Taper Method based on (c) original and (d) reconstructed time-series. Time-series length: 99, confidence intervals: red (99%), blue (95%) and green (90%). (c) and (d) both show a significant frequency of 0.16 (1/yr), which is translates to a 6-year signal. See Appendix 2 for results of frequency analysis of all analysed shells.

4   Discussion

Since modern specimens of the ocean quahog *A. islandica* cannot be found in the Mediterranean Sea nowadays, it is impossible to exclude the possibility that the found *A. islandica* shells belong to an unknown subspecies with different environmental requirements. Temperature reconstruction based on stable oxygen isotopes ($\delta^{18}O_{shell}$) are based on the equilibrium of $\delta^{18}O_{shell}$ and $\delta^{18}O_{water}$. A subspecies could potentially accreted oxygen isotope differently, e.g. no equilibrium between water and shell. Additionally, vital effects could have been played a role. However, due to a lack of DNA in fossil shells, genetic analysis cannot be performed to check this hypothesis. Since no external evidences indicate that collected shells are not *A. islandica* they can confidently be used for stable oxygen isotope ($\delta^{18}O_{shell}$) measurements and palaeo-temperature reconstructions.

Due to higher water temperatures *A. islandica* got extinct in the Mediterranean Sea more than 9,800 years ago (Dahlgren et al., 2000) and nowadays its southern distribution is limited by high water temperatures. This is controlled by thermic requirements of gametogenesis preferring mean bottom water temperatures of ≤5-6°C (Raffi, 1986).

4.1   Correlation between carbon and oxygen isotopes?

Stable carbon isotopes ($\delta^{13}C_{shell}$) are thought to be proxies for the oceanic Suess effect, sea ice cover or primary productivity, e.g. phytoplankton blooms (Schöne, 2013). However, bivalves metabolise carbon which can also be accreted in their shells. This explains the low number of stable carbon isotope ($\delta^{13}C_{shell}$) studies. Moreover, $\delta^{13}O_{shell}$ of other bivalve species are affected by ontogenetic trends (Elliot et al., 2003; Gilikin et al., 2006). But according to Schöne et al. (2005c, 2011) those age related shifts in stable carbon isotopes do not exist. Rather distinct seasonal and decadal oscillations related to phytoplankton and large-scale ocean dynamics were found (Schöne et al., 2005a). If stable carbon isotopes $\delta^{13}C_{shell}$ can be used as a robust proxy for palaeo-DIC (dissolved inorganic carbon) as suggested by Schöne et al. (2011) has to be critically examined.

The calculated correlation coefficients R^2 indicate that there is no significant correlation between stable carbon ($\delta^{13}C_{shell}$) and oxygen isotopes ($\delta^{18}O_{shell}$) (Tab. 4, Appendix 1). Carbon isotopes ($\delta^{13}C_{shell}$) show a similar annual cycle as stable oxygen isotopes ($\delta^{18}O_{shell}$) (Fig. 10). Krantz (1990) observed trends in stable carbon isotopes ($\delta^{13}C_{shell}$) profiles suggestive of spring phytoplankton blooms and summer water-column stratification in fossil scallop shells from the Pliocene and early Pleistocene collected in the Middle Atlantic Coastal Plain. This phenomenon highlights the possibilities of stable carbon isotope ($\delta^{13}C_{shell}$) studies. However, no relation between changes in phytoplankton abundance and hence in carbon isotopes can be established in measured stable carbon isotopes (see Fig.10). Another possible explanation

for seasonality in $\delta^{13}C_{shell}$ is a change in environmental factors such as river discharge (Khim et al., 2003).

4.2 Palaeo-temperatures

This study presents the first reconstructed palaeo-temperatures and seasonality of the western Mediterranean Sea during the Middle Pleistocene. Obtained temperatures correspond well with the preferred temperature range of modern *A. islandica*, which tolerates water temperatures of 0° to 20°C with an optimum from 6° to 16°C (Golikov and Scarleto, 1973). Larval development requires temperatures from 10° to 16°C (Lutz et al., 1982). Biostratigraphic analyses suggest that the *A. islandica* specimens collected on Sicily inhabit depth below the wave base level at around 20-50 m deep (pers. comm. Daniele Scarponi) corresponding well with the water depth of 5 m to more than 500 m in which *A. islandica* can be found nowadays (Schöne et al., 2013).

Palaeo-temperature reconstructions suggest a seasonal variation of ~3.0°C, indicating a low seasonality scenario. Recorded average temperature is 9.1°C (estimated $\delta^{18}O_{water}$ 0.8‰, $\delta^{18}O_{water}$ 0.9‰: 9.5°C and $\delta^{18}O_{water}$ 1.0‰: 10.0°C) and thus 10°C colder than the modern mean water temperature of the Mediterranean Sea, which is around 20-21°C (20.7°C for surface and 20.8°C for upper layer, AIS-94 in (Warn-Varnas, 1999)). Present day sea surface temperatures around Sicily range between 24°C in summer and 14°C in winter, having an annual average temperature of about 19°C (and giving a seasonality of 10°C; Hayes et al, 2005). Consequently, calculated seasonalities as well as reconstructed minimum and maximum palaeo-temperatures are lower than modern water temperatures in the Mediterranean Sea.

The $\delta^{18}O_{shell}$ values of most of the shells are within a similar range (Fig. 10). The average $\delta^{18}O_{shell}$ for the Sicilian specimens is 3.2‰ whereas two shells clearly show a lower (2.1 ‰ in shell VL-Siz-47) and higher (4.1‰ in shell VL-Siz-58) value, respectively. Since those values are not measured within one shell and the outcrop represents a time-span of 300,000 years these shells probably did not live at the same time. Comparison between the three different outcrops hints no significant differences between Middle (Rome) and Southern Italy (Sicily, Lecce). The two extrema of $\delta^{18}O_{shell}$ amplitude (Tab. 4) are represented by Sicilian shells. The $\delta^{18}O_{shell}$ values of the two shells from Rome (3.5‰) and Lecce (3.4‰) are on the Sicilian (3.2‰) average hence results indicate that water temperatures around Rome, Lecce and Sicily were at the same level in the Mediterranean Sea during the Middle Pleistocene. However, this result is just based on three measured ontogenetic years and further $\delta^{18}O_{shell}$ measurements should be conducted to verify this assumption.

The $\delta^{18}O_{shell}$ analysis of the boreal guest *A. islandica* and the reconstructed palaeo-water temperatures indicate a low seasonality scenario (~3°C) that is contrary to previous

assumptions that the simultaneous occurrence of warm-water and boreal species in the Mediterranean Sea during the middle Calabrian can be explained by a high-seasonality scenario (10°C) (e.g. Raffi, 1986; Crippa et al., 2016). However, a high seasonality scenario is mainly assumed for the first phase of the migration of boreal guests in the Santernian substage of the Lower Pleistocene Mediterranean (Raffi, 1986), whereas shells considered in this study are from the Sicilian substage. Raffi (1986) highlighted that the colonization success of *A. islandica* is more likely linked to cold winter water temperatures than to cooler summer temperatures. According to the described high seasonality scenario summer surface water temperatures not lower than 19 to 20°C and winter temperatures around 9 to 10°C could explain the simultaneous occurrence of boreal and warm-water species.

The lack in seasonality shown by stable oxygen isotope data ($\delta^{18}O_{shell}$) and the amount of boreal guests suggest that the Sicilian outcrop represents a maximum glacial phase (pers. comm. Daniele Scarponi), which is supported by the low reconstructed palaeo-temperatures. The increasing diversity of boreal guests in the upper Emillian and upper Sicilian (substages of the Calabrian) suggest a further drop in temperature over time (Raffi, 1986). Moreover, the maximum diversity of boreal guests recorded in the Sicilian substage may coincide with the beginning of the "first major Northern Hemisphere glaciation of Middle Pleistocene character" (Shackleton and Opdyke, 1976). Both support the assumption that the collected shells represent a maximum glacial phase.

However, Crippa et al. (2016) showed that the Palaeo-Adriatic was characterised by a high seasonality (~10°C) and low winter temperatures (0.8-1.6°C), which correspond well with the assumption of Raffi (1986). Modern temperature conditions in the Mediterranean Sea are by far too warm for boreal species (mean water temperature of the Mediterranean Sea (depth 20-50m) 20-21°C; Warn-Varnas, 1999), such as *A. islandica*, and clearly indicate that different climatic conditions prevailed in the Middle Pleistocene. Reconstructed water temperatures suggest minimum temperatures of 0.8 to 1.6°C with a high seasonal temperature variation of 8.7 to 10.9°C in the Palaeo-Adriatic Sea (Crippa et al., 2016). Reconstructed water temperatures and the conclusion that the Palaeo-Adriatic was characterized by high seasonality differ from the results obtained in shells from the Paleo-Mediterranean Sea around Sicily. However, also the modern Northern Adriatic Sea represents the region of the Mediterranean with the highest seasonality and minimum winter temperatures (Raffi, 1986). The ontogenetic ages of the Arda shells is not known (Crippa et al., 2016). High ontogenetic ages could imply that the high variabilities of $\delta^{18}O_{shell}$ values are not influenced by strong salinity fluctuations. The Arda section represents the time-span from 1.9 to 1.2 Ma (Crippa et al., 2016), while the Sicilian outcrop is covering the middle Calabrian from 1.2 to 0.9 Ma. Additionally, nowadays as well as in the Pleistocene, latitudinal gradients exist in vegetation

and climate throughout the Mediterranean region (Nebout and Grazzini, 1991), which could explain the deviating palaeo-temperature reconstructions around Sicily and the further north located Arda section. The western basin (Sicily) became significantly cooler than the eastern basin (Adriatic Sea) in the Middle Pleistocene coinciding with the intensification of glacial/ interglacial oscillation (Thunell, 1979b). The reconstructed palaeo-temperatures in this study are significantly colder than those prevailing in the Mediterranean Sea nowadays (mean water temperature of 20-21°C) and show a low seasonality scenario (~3°C) contrary to Crippa et al. (2016).

However, $\delta^{18}O_{shell}$ represents a proxy, which is influenced by both temperature and salinity changes (i.e. $\delta^{18}O_{water}$). Strong salinity changes would obscure seasonal temperature variation even though climate might have been characterized by high seasonality (Schöne, 2013). The bivalve *A. islandica* tolerates salinity ranges from 22 to 35 PSU (Winter, 1969; Oeschger and Storey, 1993) and has regulation mechanisms to cope with those changes. However, strong salinity changes represent a stress factor. This assumption is yet contradicted by the relative high ontogenetic ages (>300 years) observed in the *A. islandica* specimens of this study. It is known that unfavourable and strongly variable salinity conditions reduce the life span of *A. islandica* as it can be observed in the Baltic Sea (Zettler et al., 2001).

4.3 Palaeoceanography: support of low seasonality scenario?

According to biostratigraphic analysis collected shells belong to the Calabrian, Middle Pleistocene (Section 2.1). Subtropical foraminiferal species (*Globigerina guadrilobatus*, *G. obliquus* and *G. rubens*) dominated the early Pliocene assemblages and were replaced by cold-water species ("boreal guests") during the long-term cooling of the Pliocene and Pleistocene (Thunell et al., 1991). Cold currents from the North Atlantic entering the Mediterranean Sea also contributed to the cooling at that time (Becker et al., 2006). The increase of the Northern Hemisphere ice sheet and the intensification of glacial episodes marked the Mediterranean climate during the Pleistocene (Sosdian and Rosenthal, 2009). The Early-Middle Pleistocene Transition (EMPT) was characterized by increasing severity and duration of cold stages that coincided with the increasing global ice volume. These climate changes support the assumption that a maximum glacial phase prevailed in the Mediterranean Sea during the Middle Pleistocene (Calabrian). During the last glacial maximum (LGM; 18,000 years ago), surface temperatures in the Strait of Sicily were ~17°C in glacial summers and cooled down to 7-10°C in glacial winters (Hayes et al., 2005). These temperatures are higher than reconstructed water temperatures of the Mediterranean Sea during the Middle Pleistocene (Fig. 11) which suggestive represent a maximum glacial phase. This points out the strength of the maximum glacial phase of the Calabrian, Middle Pleistocene, compared to the LGM. Benthic foraminiferal oxygen isotope ($\delta^{18}O_{shell}$) data revealed that the Mid-

Pleistocene transition (MPT) led to a transition from 41 ky cycles to 100 ky cycles in sea-ice dynamics. This is associated with orbital cycles that have an effect on seasonality (Sosdian and Rosenthal, 2009). According to Thunell (1979b) the Mediterranean Sea had water temperature oscillations of 2-3°C in the early Pleistocene that increased up to 5-6°C in the late Pleistocene. Palaeoceanography is mostly in accordance with obtained data that show a low-seasonality scenario (~3°C; Figs 10&11). But palaeo-oceanographic studies usually cover time-spans of several thousands to several hundreds of thousands of years, sometimes even millions (e.g. sediment cores), and do not contain information of temperature variation on small time-scales, i.e. intra-annual seasonal variation (e.g. Thunell, 1979b).

4.4 *Multi-year signals in the Pleistocene Mediterranean Sea*

Annual and daily growth patterns are formed by variable growth rates over the year or the day (Schöne et al., 2013). Those daily and annual growth increments represent time slices of approximately the same duration and can be placed in a precise chronological context (Schöne, 2013). However, growth patterns of *A. islandica* show an ontogenetic growth trend, which is removed by calculating the dimensionless standardized growth index. Annual increment widths increase during the first years, reach a maximum between age five to ten and then decrease gradually (Schöne, 2013). Thereby growth is mainly controlled by water temperature and food availability (Witbaard et al., 1999).

Schöne et al. (2003) and Butler et al. (2010) could identify multi-year, multi-decadal to centennial-scale oscillations in master chronologies of *A. islandica* and associated those to the NAO, the Hale and Schwabe solar cycle, the Atlantic Multidecadal Oscillation (AMO) and the de Vries solar cycle. Time-series of fossil Mediterranean *A. islandica* shells show a clear ~6-year cycle ranging between 5 and 7 years (Section 3.6; Fig. 12). Apparently, temperature or food supply have changed on a multi-year scale. The effect of temperature on shell growth is relatively small (Schöne, 2013) whereas food supply as well as food quality (Witbaard, 1996) are important triggers of shell growth. Therefore, it is likely to assume that food availability and food quality might have been influenced by multi-year changes in climate and environmental conditions during the Middle Pleistocene. Witbaard et al. (2003) linked shell growth of *A. islandica* to copepod density. Downward flux of particles was reduced by large copepod population and caused a decrease in shell growth rates. In turn, copepod density decreases with a decreasing phytoplankton (primary productivity) population. These changes in food supply may be associated by variation of NAO states (Schöne, 2013). Increased resuspension of organic particles may stimulate growth rates since, according to Schöne (2013), a maximal pressure level difference between Iceland (Low) and Azores (High), i.e. during positive NAO years (Schöne et al. 2003) favors wind-driven-mixing of water masses. These particles act as a food source for bivalves. This phenomenon results in higher amounts of resuspendend

organic particles at the sea floor in the North Sea. However, similar scenarios are also possible in the Mediterranean Sea but they have to be further investigated. The prominent ~6-year signal in shell growth may be linked to the NAO since this oscillation has a 5-9 year cycle (Schöne et al., 2003; Schöne, 2013). The NAO index is often used to express climatic variability in Europe (Martin et al., 2012). Since the so called Mediterranean Oscillation (MO) is also known to exist in the Mediterranean Sea, it should be checked if this oscillation can explain the ~6-year cyclicity. The MO is highly linked to both the NAO as well as the Arctic Oscillation (AO) (Martín-Vide and Lopez-Bustins, 2006). Dünkeloh and Jacobeit (2003) firstly establish the MO, which has a periodicity of 5 and 22 years and statistically non-significant peaks of 2 and 8 years. This does, however, not explain the found ~6-year signal. The MO is based on atmospheric dynamics between the eastern and western sub-basin of the Mediterranean Sea and influences rainfall. However, the dipole of the MO covers the north-western sector of the Mediterranean Sea, i.e. mostly the Iberian Peninsula being responsible for precipitation in the Gulf of Valencia or the Bay of Biscay depending on positive or negative MO modes (Martín-Vide and Lopez-Bustins, 2006). Since the shells of this study were collected in Middle and Southern Italy, the Mediterranean Oscillation may not be the driving factor that explains the 6-year signal, which was found in multiple time-series.

Due to the assumption that the studied shells lived over a time period of several thousands of years and considering the fact that the 6-year oscillation was found in the growth pattern of multiple fossil shells suggests that this multi-year signal did occur over a longer time period at varying or stable intensities and maybe even on a larger, supra-regional scale. However, it remains challenging to link differences in shell growth to modern atmospheric and climate oscillations. Climate conditions during the Pleistocene differed significantly from modern conditions as suggested by the stable oxygen isotope ($\delta^{18}O_{shell}$) data (see Section 3.3, Fig. 10). This also leaves the possibility that the observed periodicity of past climatic and environmental signals may have changed or that the observed signal (of unknown origin) has been unique for the Pleistocene and does not exist nowadays. In this study signals with a periodicity of 2 to 3 years are not further investigated. In general, biannual signals can be found in many climatic time-series and are challenging to link to certain oscillation patterns.

5   Conclusions

I present a palaeo-environmental reconstruction for the middle Calabrian in the Mediterranean Sea, based on fossil *A. islandica* shells from Pleistocene successions in Middle and Southern Italy. According to preliminary biostratigraphic analysis the outcrops have geological ages of 1.2 to 0.9 Ma for the Sicily outcrop and 1.2 to 1.4 Ma for Rome and Lecce, respectively.

- Stable oxygen isotope ($\delta^{18}O_{shell}$) profiles of eleven fossil *A. islandica* shells show a relatively low seasonality. $\delta^{18}O_{shell}$ amplitude values vary between 0.4‰ and 1.1‰ implying a reconstructed water temperature amplitude between 1.7°C and 4.8°C (Section 3.3, Tab. 3, Fig. 10).

- The reconstructed average water temperature for the Sicilian population is 9.1°C ($\delta^{18}O_{water}$ 0.8‰; $\delta^{18}O_{water}$ 0.9‰: 9.5°C and $\delta^{18}O_{water}$ 1.0‰: 10.0°C) and coincides well with temperature requirements for modern *A. islandica* (Section 3.5, Tab. 5, Fig. 11).

- The low seasonality scenario (~3°C) represented by the shells and the low water temperatures (around 10°C colder than modern water temperatures around Sicily) conclude that the shells represent a maximum glacial phase with relatively constant water temperatures throughout the year (Section 3.3, Tabs 3&5, Figs 10&11).

- A prominent ~6-year signal was found in fossil *A. islandica* shells by means of frequency analysis, which may be linked to the NAO (Section 3.6, Fig. 12). Due to their longevity *A. islandica* shells are well applicable for frequency analysis (maximum ontogenetic age >300 years) (Section 3.3, Tab. 2, Appendix 2).

- *A. islandica* shells from the Middle Pleistocene are actual well suited for environmental and climate reconstructions on different time-scales, seasonal and multi-year (Section 3.3, 3.5 and 3.6, Tabs 2,3&5, Figs 10,11&12).

- Confocal Raman microscopy showed that fossil shells from these three Pleistocene outcrops in Middle and Southern Italy are well preserved since they do not show potential diagenetic alterations. Therefore, they can be used for geochemical as well as for anatomical analyses (shell growth rate) (Section 3.2, Fig. 9).

Climate archives such as the bivalve *A. islandica* are well suited for the reconstruction of palaeo-environmental and palaeo-oceanographic conditions. However, it will remain almost impossible to provide continuous master chronologies for the Pleistocene. The studied outcrops represent time periods of 200,000 to 300,000 years implying that it is very unlikely that the collected shells lived at the same time or in over-lapping years. Even so, this study highlights the potential of individual shell chronologies.

6   Outlook

The stable oxygen isotope ($\delta^{18}O_{shell}$) data of fossil *A. islandica* shells in this study suggest a low seasonality scenario (~3°C). This is in sharp contrast to previous assumptions that the simultaneous occurrence of boreal and warm-water species in the Mediterranean Sea during the Pleistocene can be explained by high seasonality (~10°C). Furthermore, this study reveals a prominent 6-year cyclicity within the growth pattern of most bivalves. To deepen the knowledge about the Pleistocene climate in the Mediterranean Sea and to verify the results, I suggest investigating the following aspects in further studies:

- The high abundance of boreal guests found in the studied outcrops and the fact that some species still occur in the Mediterranean Sea while others (e.g. *A. islandica*) got extinct indicate that the climate in the Mediterranean Sea during the Pleistocene differed from modern conditions. Studying the temperature range of collected associated fauna may indicate the temperature range of past climate conditions and give further hints about the seasonal temperature variation.

- Further information about the outcrops obtained by biostratigraphic analysis (including water depth) would help to get a more detailed picture of the environmental conditions in which *A. islandica* and associated fauna lived.

- To verify the assumed low seasonality scenario, I suggest further stable oxygen isotope ($\delta^{18}O_{shell}$) measurements in other suited species (e.g. *Glycymeris* sp.), which were also collected in the studied outcrops. Obtained results can be compared and linked with presented results of fossil *A. islandica* shells. Moreover, further stable oxygen isotope ($\delta^{18}O_{shell}$) measurements should be conducted on shells from Rome and Lecce since this study focuses on the Sicilian specimens.

- Frequency analysis revealed a prominent 6-year signal. I suggest performing frequency analysis on growth records of additional species found in the studied outcrops (e.g. *Glycymeris* sp.) to check if this signal can also be found in other species.

- Some shells could not be included in the frequency analysis since annual growth shell increments were not continuously visible. Further visualization techniques (e.g. Mutvei's solution or mapping by confocal Raman microscopy) should be tested.

References

Becker, J., Lourens, L. J., & Raymo, M. E. (2006). High-frequency climate linkages between the North Atlantic and the Mediterranean during marine oxygen isotope stage 100 (MIS100). *Paleoceanography*, *21*(3).

Bethoux, J. P., Gentili, B., Morin, P., Nicolas, E., Pierre, C., & Ruiz-Pino, D. (1999). The Mediterranean Sea: a miniature ocean for climatic and environmental studies and a key for the climatic functioning of the North Atlantic. *Progress in Oceanography*, *44*(1), 131-146.

Brand, U., Logan, A., Bitner, M. A., Griesshaber, E., Azmy, K., & Buhl, D. (2011). What is the ideal proxy of Palaeozoic seawater chemistry?. *Memoirs of the Association of Australasian Palaeontologists*, (41), 9.

Butler, P. G., Richardson, C. A., Scourse, J. D., Wanamaker, A. D., Shammon, T. M., & Bennell, J. D. (2010). Marine climate in the Irish Sea: analysis of a 489-year marine master chronology derived from growth increments in the shell of the clam Arctica islandica. *Quaternary Science Reviews*, *29*(13), 1614-1632.

Butler, P. G., Wanamaker, A. D., Scourse, J. D., Richardson, C. A., & Reynolds, D. J. (2013). Variability of marine climate on the North Icelandic Shelf in a 1357-year proxy archive based on growth increments in the bivalve Arctica islandica. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *373*, 141-151.

Crippa, G., Angiolini, L., Bottini, C., Erba, E., Felletti, F., Frigerio, C., ... & Raineri, G. (2016). Seasonality fluctuations recorded in fossil bivalves during the early Pleistocene: Implications for climate change. *Palaeogeography, Palaeoclimatology, Palaeoecology*.

Dahlgren, T. G., Weinberg, J. R., & Halanych, K. M. (2000). Phylogeography of the ocean quahog (Arctica islandica): influences of paleoclimate on genetic diversity and species range. *Marine Biology*, *137*(3), 487-495.

Dettman, D. L., & Lohmann, K. C. (1995). Microsampling Carbonates for Stable Isotope and Minor Element Analysis: Physical Separation of Samples on a 20 Micrometer Scale: RESEARCH METHOD PAPER. *Journal of Sedimentary Research*, *65*(3).

Dettman, D. L., Reische, A. K., & Lohmann, K. C. (1999). Controls on the stable isotope composition of seasonal growth bands in aragonitic fresh-water bivalves (Unionidae). *Geochimica et Cosmochimica Acta*, *63*(7), 1049-1057.

Dünkeloh, A., & Jacobeit, J. (2003). Circulation dynamics of Mediterranean precipitation variability 1948–98. *International Journal of Climatology*, *23*(15), 1843-1866.

Elliot, M., Welsh, K., Chilcott, C., McCulloch, M., Chappell, J., & Ayling, B. (2009). Profiles of trace elements and stable isotopes derived from giant long-lived Tridacna gigas bivalves: potential applications in paleoclimate studies. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *280*(1), 132-142.

Ericson, D. B., & Wollin, G. (1968). Pleistocene climates and chronology in deep-sea sediments. *Science, 162*(3859), 1227-1234.

Fusco, F. (2007). Vegetation response to early Pleistocene climatic cycles in the Lamone valley (Northern Apennines, Italy). *Review of Palaeobotany and Palynology*, *145*(1), 1-23.

Gillikin, D. P., Hutchinson, K. A., & Kumai, Y. (2009). Ontogenic increase of metabolic carbon in freshwater mussel shells (Pyganodon cataracta). *Journal of Geophysical Research: Biogeosciences*, *114*(G1).

Giorgi, F. (2006). Climate change hot-spots. *Geophysical research letters*,*33*(8).

Giorgi, F., & Lionello, P. (2008). Climate change projections for the Mediterranean region. *Global and Planetary Change*, *63*(2), 90-104.

Golikov, A. N., & Scarlato, O. A. (1973). Method for indirectly defining optimum temperatures of inhabitancy for marine cold-blooded animals.*Marine Biology*, *20*(1), 1-5.

Grossman, E. L., & Ku, T. L. (1986). Oxygen and carbon isotope fractionation in biogenic aragonite: temperature effects. *Chemical Geology: Isotope Geos*

Hansen, J. W., Mason, S. J., Sun, L., & Tall, A. (2011). Review of seasonal climate forecasting for agriculture in sub-Saharan Africa. *Experimental Agriculture*, *47*(2), 205.

Hayes, A., Kucera, M., Kallel, N., Sbaffi, L., & Rohling, E. J. (2005). Glacial Mediterranean Sea surface temperatures based on planktonic foraminiferal assemblages. *Quaternary Science Reviews*, *24*(7), 999-1016.

Hickson, J. A., Johnson, A. L., Heaton, T. H., & Balson, P. S. (1999). The shell of the Queen Scallop Aequipecten opercularis (L.) as a promising tool for palaeoenvironmental reconstruction: evidence and reasons for equilibrium stable-isotope incorporation. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *154*(4), 325-337.

Howell, M. W., Rio, D., & Thunell, R. C. (1990). Laminated sediments from the Vrica Section (Calabria, S. Italy): evidence for Plio-Pleistocene climatic change in the Mediterranean region. *Palaeogeography, palaeoclimatology, palaeoecology*, *78*(3-4), 195-216.

International Commission on Stratigraphy. (n.d.). Retrieved May 17, 2016, from http://www.stratigraphy.org/.

IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535.

Ivany, L. C., Brey, T., Huber, M., Buick, D. P., & Schöne, B. R. (2011). El Niño in the Eocene greenhouse recorded by fossil bivalves and wood from Antarctica. *Geophysical Research Letters*, *38*(16).

Khim, B. K., Krantz, D. E., Cooper, L. W., & Grebmeier, J. M. (2003). Seasonal discharge of estuarine freshwater to the western Chukchi Sea shelf identified in stable isotope profiles of mollusk shells. *Journal of Geophysical Research: Oceans*, *108*(C9).

Krantz, D. E. (1990). Mollusk-isotope records of Plio-Pleistocene marine paleoclimate, US middle Atlantic coastal plain. *Palaios*, 317-335.

Luterbacher, J. (2006). Mediterranean climate variability over the last centuries: a review. *The Mediterranean climate: an overview of the main characteristics and issues 4*, p-27.

Lutz, R. A., Mann, R., Goodsell, J. G., & Castagna, M. (1982). Larval and early post-larval development of Arctica islandica. *Journal of the Marine Biological Association of the United Kingdom*, *62*(04), 745-769.

Maliva, R. G. (1998). Skeletal aragonite neomorphism—quantitative modelling of a two-water diagenetic system. *Sedimentary Geology*, *121*(3), 179-190.

Martín, P., Sabatés, A., Lloret, J., & Martin-Vide, J. (2012). Climate modulation of fish populations: the role of the Western Mediterranean Oscillation (WeMO) in sardine (Sardina pilchardus) and anchovy (Engraulis encrasicolus) production in the north-western Mediterranean. *Climatic Change*, *110*(3-4), 925-939.

Martin-Vide, J., & Lopez-Bustins, J. A. (2006). The western Mediterranean oscillation and rainfall in the Iberian Peninsula. *International Journal of Climatology*, *26*(11), 1455-1475.

Nebout, N. C., & Grazzini, C. V. (1991). Late Pliocene northern hemisphere glaciations: the continental and marine responses in the central Mediterranean. *Quaternary Science Reviews*, *10*(4), 319-334.

Nehrke, G., & Nouet, J. (2011). Confocal Raman microscope mapping as a tool to describe different mineral and organic phases at high spatial resolution within marine biogenic carbonates: case study on Nerita undata (Gastropoda, Neritopsina). *Biogeosciences (BG)*, *8*(12), 3761-3769.

Oeschger, R., & Storey, K. B. (1993). Impact of anoxia and hydrogen sulphide on the metabolism of Arctica islandica L.(Bivalvia). *Journal of experimental marine biology and ecology*, *170*(2), 213-226.

Raffi, S. (1986). The significance of marine boreal molluscs in the early Pleistocene faunas of the Mediterranean area. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *52*(3-4), 267-289.

Riedel, R. (1983). *Fauna und Flora des Mittelmeeres*, Hamburg and Berlin, Germany. Paul Perey.

Royer, C., Thébault, J., Chauvaud, L., & Olivier, F. (2013). Structural analysis and paleoenvironmental potential of dog cockle shells (Glycymeris glycymeris) in Brittany, northwest France. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *373*, 123-132.

Schöne, B. R., Oschmann, W., Rössler, J., Castro, A. D. F., Houk, S. D., Kröncke, I., ... & Dunca, E. (2003). North Atlantic Oscillation dynamics recorded in shells of a long-lived bivalve mollusk. *Geology*, *31*(12), 1037-1040.

Schöne, B. R., Castro, A. D. F., Fiebig, J., Houk, S. D., Oschmann, W., & Kröncke, I. (2004). Sea surface water temperatures over the period 1884–1983 reconstructed from oxygen isotope ratios of a bivalve mollusk shell (Arctica islandica, southern North Sea). *Palaeogeography, Palaeoclimatology, Palaeoecology*, *212*(3), 215-232.

Schöne, B. R., Fiebig, J., Pfeiffer, M., Gleβ, R., Hickson, J., Johnson, A. L., ... & Oschmann, W. (2005a). Climate records from a bivalved Methuselah (Arctica islandica, Mollusca; Iceland). *Palaeogeography, Palaeoclimatology, Palaeoecology*, *228*(1), 130-148.

Schöne, B. R., Houk, S. D., Castro, A. D. F., Fiebig, J., Oschmann, W., Kröncke, I., ... & Gosselck, F. (2005b). Daily growth rates in shells of Arctica islandica: assessing sub-seasonal environmental controls on a long-lived bivalve mollusk. *Palaios*, *20*(1), 78-92.

Schöne, B. R., Pfeiffer, M., Pohlmann, T., & Siegismund, F. (2005c). A seasonally resolved bottom-water temperature record for the period AD 1866–2002 based on shells of Arctica islandica (Mollusca, North Sea).*International Journal of Climatology*, *25*(7), 947-962.

Schöne, B. R., Wanamaker, A. D., Fiebig, J., Thébault, J., & Kreutz, K. (2011). Annually resolved δ 13 C shell chronologies of long-lived bivalve mollusks (Arctica islandica) reveal oceanic carbon dynamics in the temperate North Atlantic during recent centuries. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *302*(1), 31-42.

Schöne, B. R. (2013). Arctica islandica (Bivalvia): A unique paleoenvironmental archive of the northern North Atlantic Ocean. *Global and Planetary Change*, *111*, 199-225.

Schrag, D. P., Adkins, J. F., McIntyre, K., Alexander, J. L., Hodell, D. A., Charles, C. D., & McManus, J. F. (2002). The oxygen isotopic composition of seawater during the Last Glacial Maximum. *Quaternary Science Reviews*,*21*(1), 331-342.

Shackleton, N. J., & Opdyke, N. D. (1976). Oxygen-isotope and paleomagnetic stratigraphy of Pacific core V28-239 late Pliocene to latest Pleistocene. *Geological Society of America Memoirs*, *145*, 449-464.

Sosdian, S., & Rosenthal, Y. (2009). Deep-sea temperature and ice volume changes across the Pliocene-Pleistocene climate transitions. *Science*,*325*(5938), 306-310.

Thórarinsdóttir, G. G., & Einarsson, S. T. (1996). Distribution, abundance, population structure and meat yield of the ocean quahog, Arctica islandica, in Icelandic waters. *Journal of the Marine Biological Association of the United Kingdom*, *76*(04), 1107-1114.

Thunell, R. C. (1979a). Pliocene-Pleistocene paleotemperature and paleosalinity history of the Mediterranean Sea: results from DSDP Sites 125 and 132. *Marine Micropaleontology*, *4*, 173-187.

Thunell, R. C. (1979b). Climatic evolution of the Mediterranean Sea during the last 5.0 million years. *Sedimentary Geology*, *23*(1-4), 67-79.

Thunell, R., Rio, D., Sprovieri, R., & Vergnaud-Grazzini, C. (1991). An overview of the post-Messinian paleoenvironmental history of the western Mediterranean. *Paleoceanography*, *6*(1), 143-164.

Ulijaszek, S. J., & Strickland, S. S. (2009). *Seasonality and human ecology*(Vol. 35). Cambridge University Press.

Wall, M., & Nehrke, G. (2012). Identification of two organic bands showing different chemical composition within the skeleton of Porites lutea: a confocal Raman microscopy study. *Biogeosciences Discussions*, *9*(7), 8273.

Warn-Varnas, A., Sellschopp, J., Haley, P. J., Leslie, W. G., & Lozano, C. J. (1999). Strait of Sicily water masses. *Dynamics of atmospheres and oceans*, *29*(2), 437-469.

Winter, J. E. (1969). Über den Einfluß der Nahrungskonzentration und anderer Faktoren auf Filtrierleistung und Nahrungsausnutzung der Muscheln Arctica islandica und Modiolus modiolus. *Marine Biology*, *4*(2), 87-135.

Witbaard, R. (1996). Growth variations in Arctica islandica L.(Mollusca): a reflection of hydrography-related food supply. *ICES Journal of Marine Science: Journal du Conseil*, *53*(6), 981-987.

Witbaard, R., Duineveld, G. C. A., & De Wilde, P. A. W. J. (1999). Geographical differences in growth rates of Arctica islandica (Mollusca: Bivalvia) from the North Sea and adjacent waters. *Journal of the Marine Biological Association of the UK*, *79*(05), 907-915.

Witbaard, R., Jansma, E., & Klaassen, U. S. (2003). Copepods link quahog growth to climate. *Journal of Sea Research*, *50*(1), 77-83.

Zettler, M. L., Bonsch, R., & Gosselck, F. (2001). Distribution, abundance, and some population characteristics of the ocean quahog, Arctica islandica (Linnaeus, 1767), in the Meeklenburg Bight (Baltic Sea). *Journal of Shellfish Research*, *20*(1), 161-170.

d) VL-Siz-39

R² = 0.0209

e) VL-Siz-41

R² = 0.0321

f) VL-Siz-47

R² = 0.0886

g) VL-Siz-55

R² = 0.1774

h) VL-Siz-56

R² = 0.0057

i) VL-Siz-58

R² = 0.0988

j) VL-Rom-61
$R^2 = 0.1419$

k) VL-Le3-3
$R^2 = 0.0107$
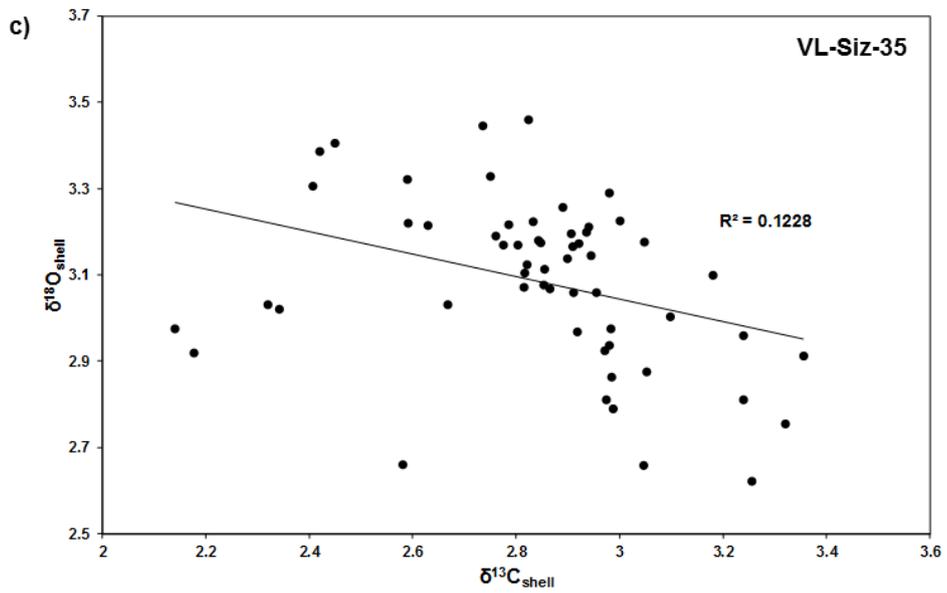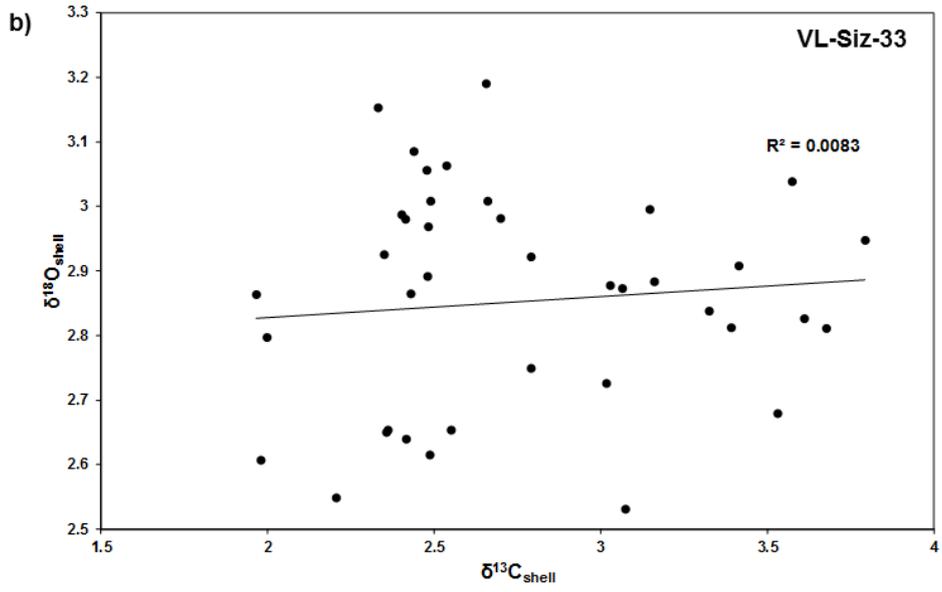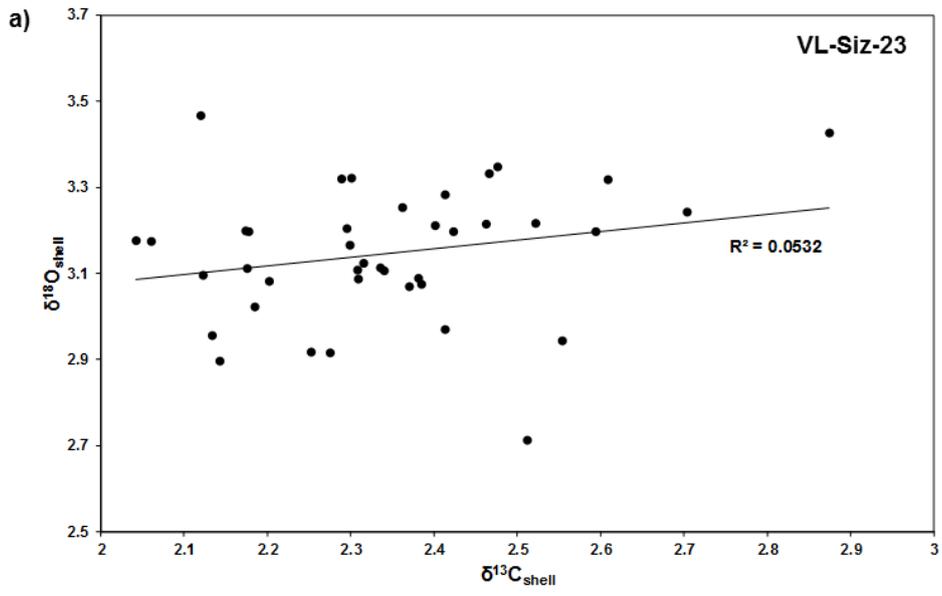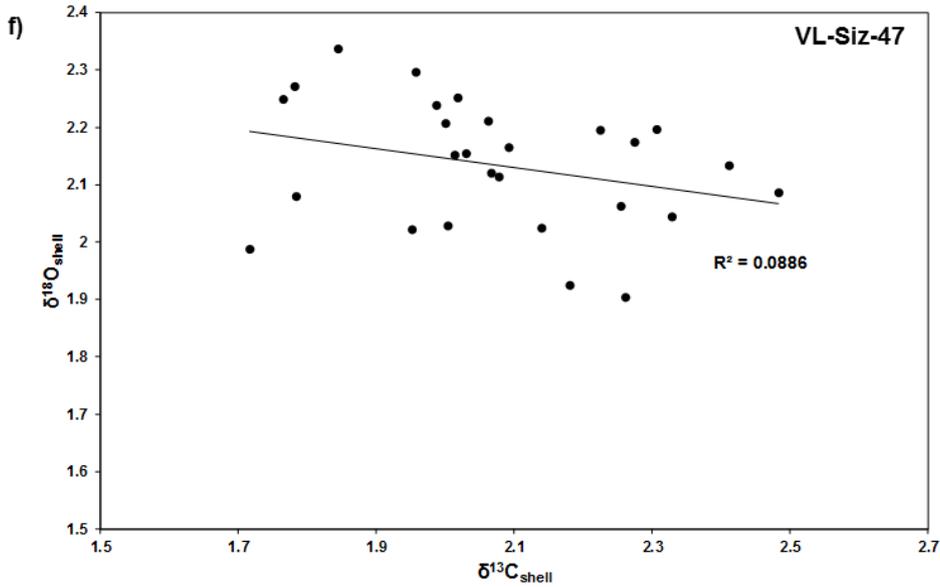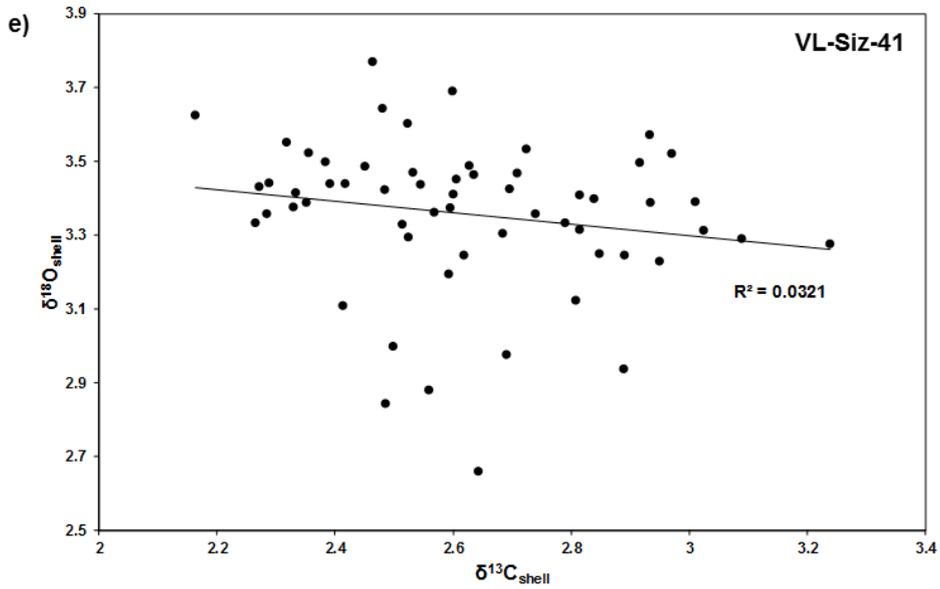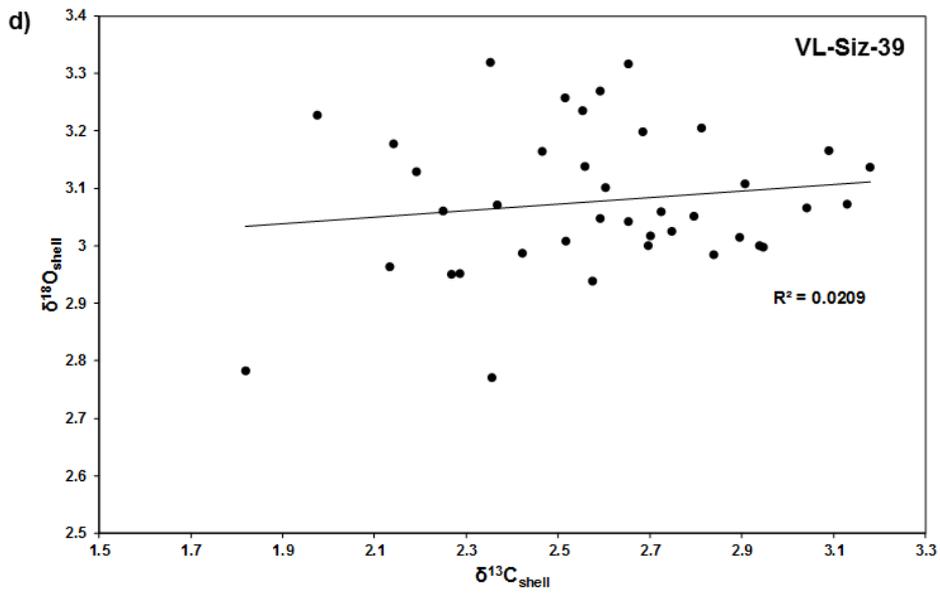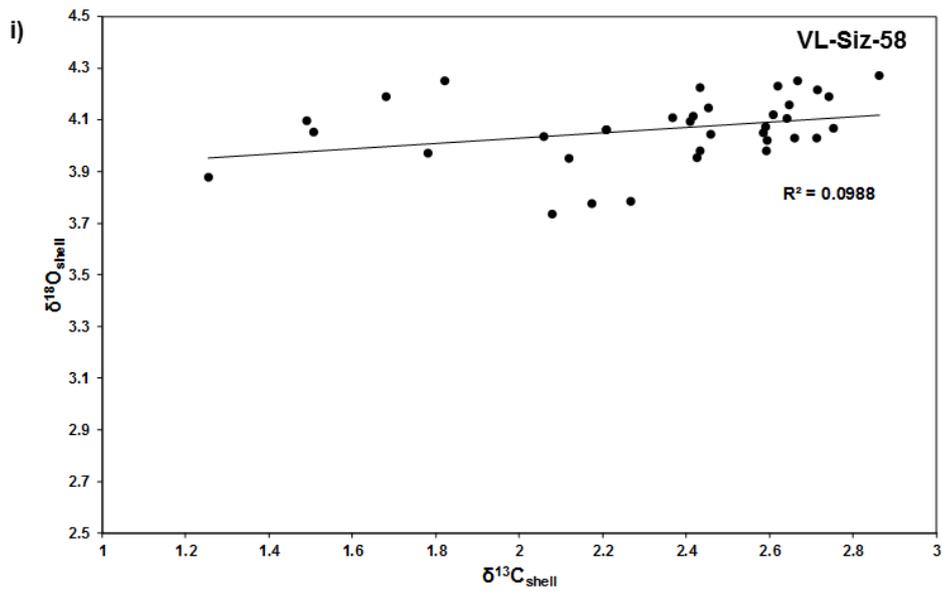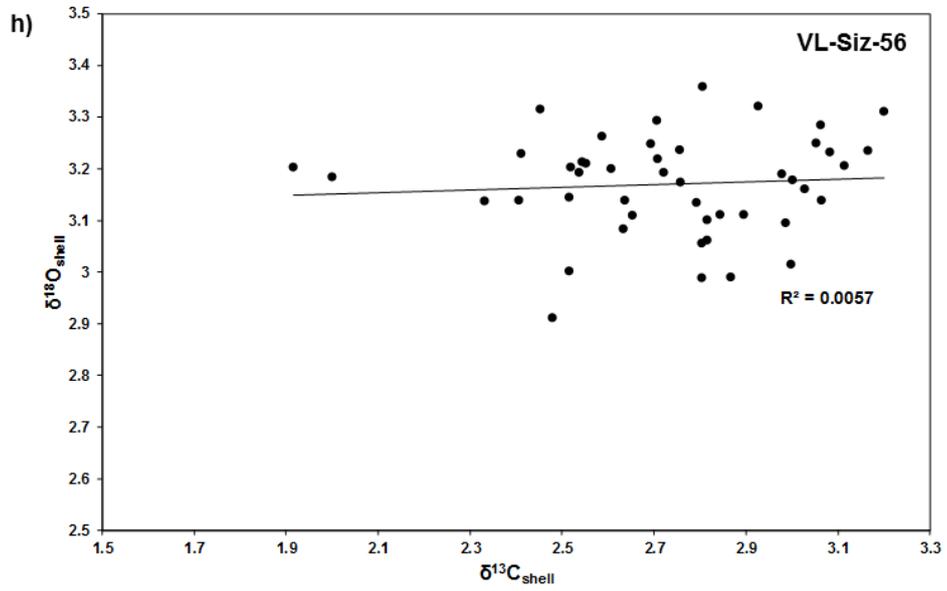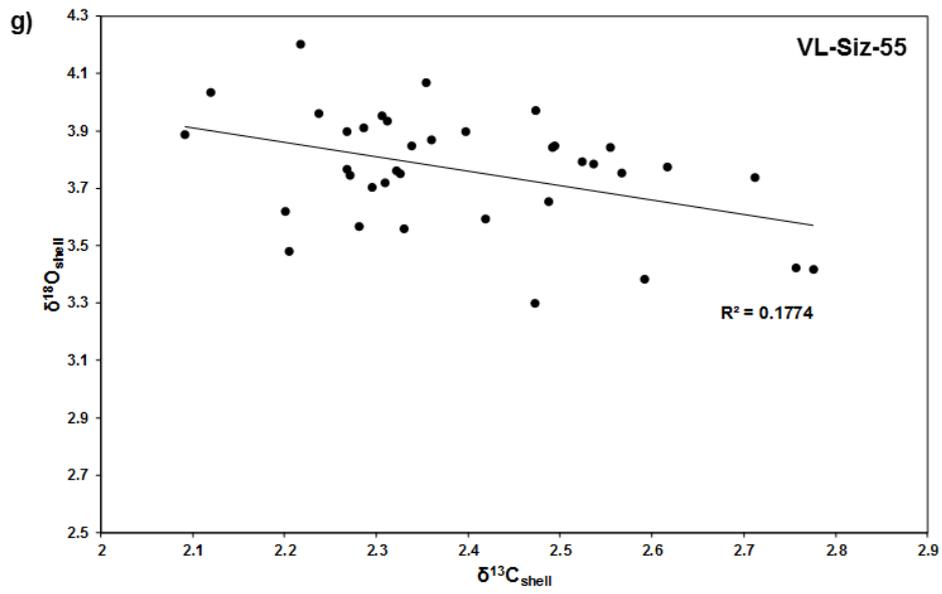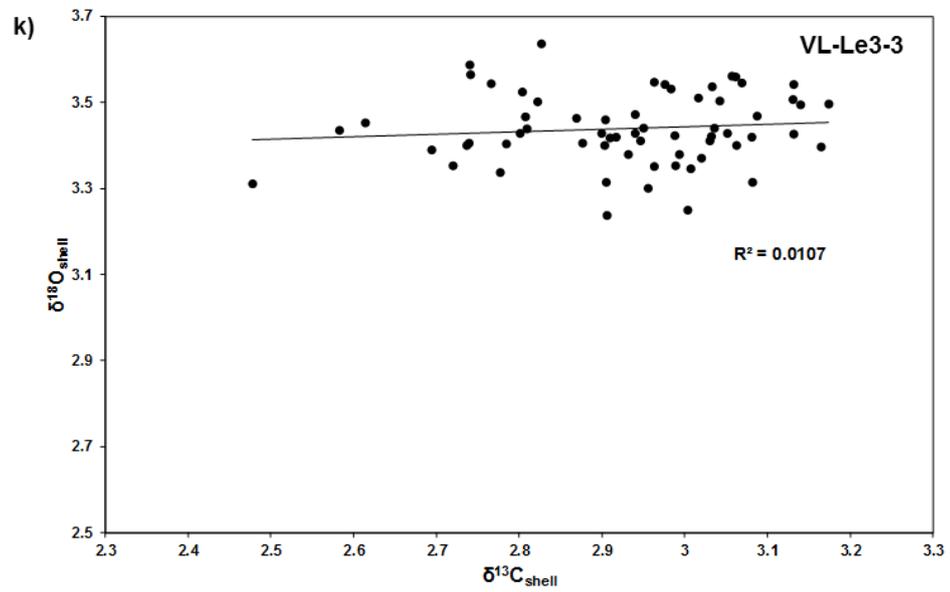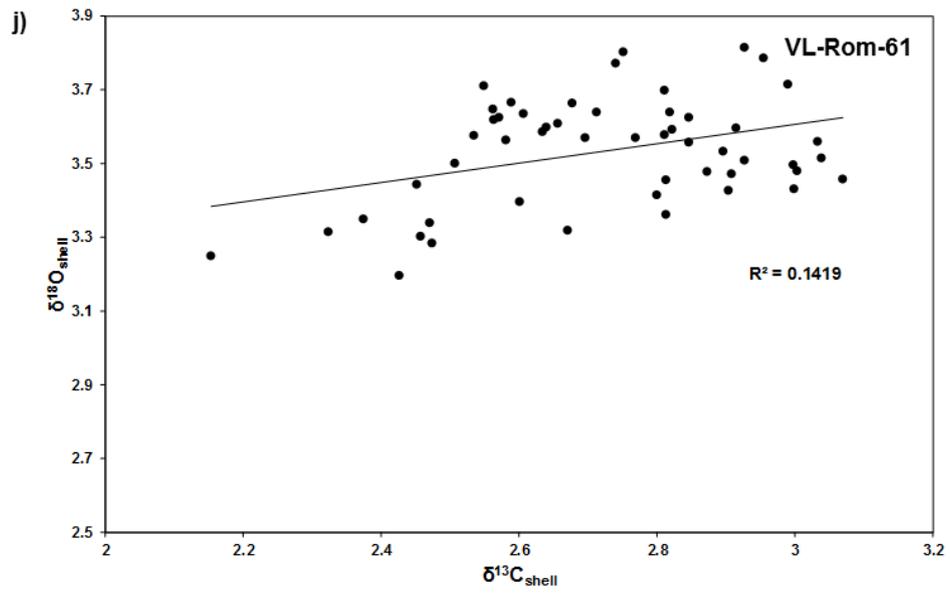
Appendix 1: Correlation between stable carbon ($\delta^{13}O$) and oxygen ($\delta^{18}O$) isotopes by means of calculated correlation coefficient R^2.
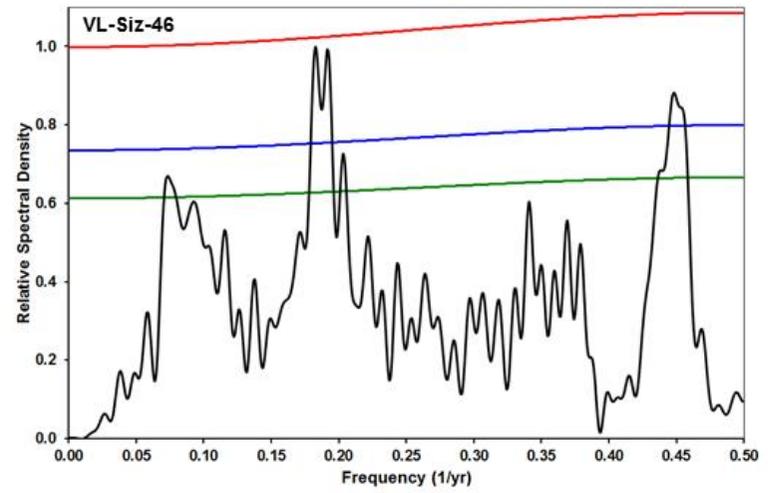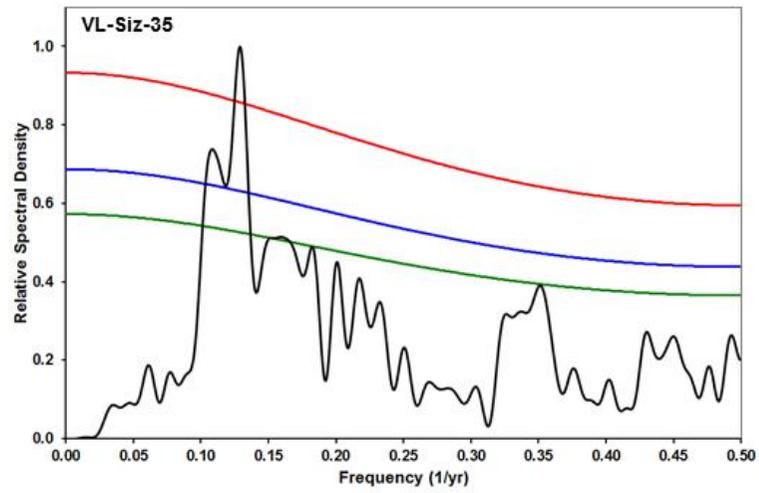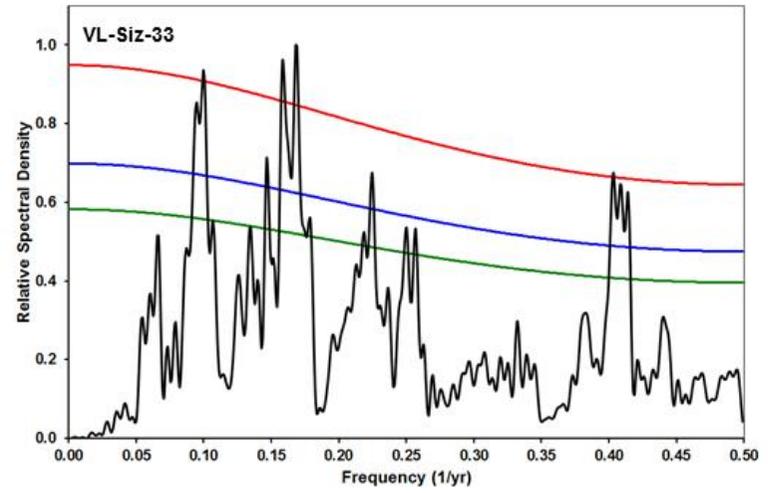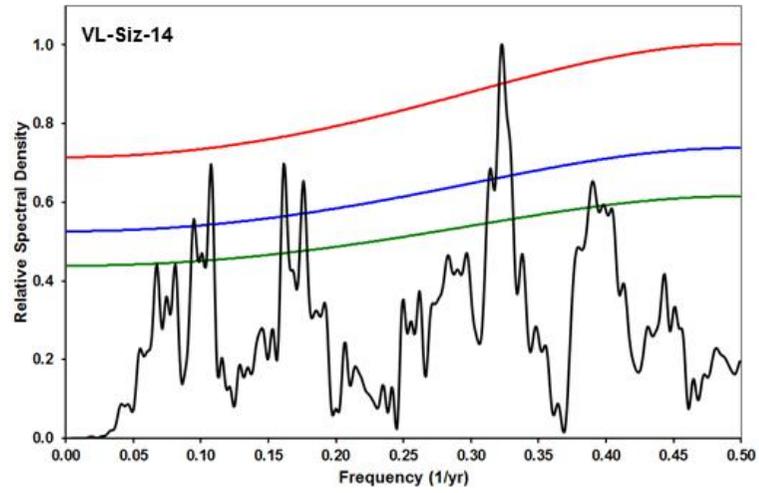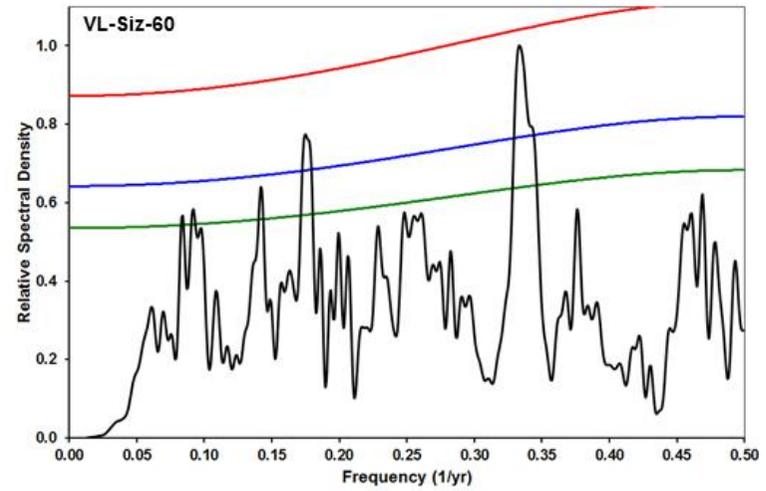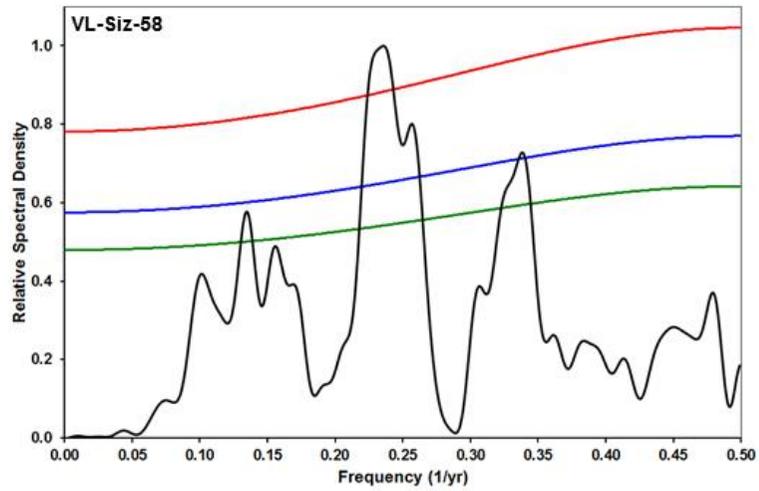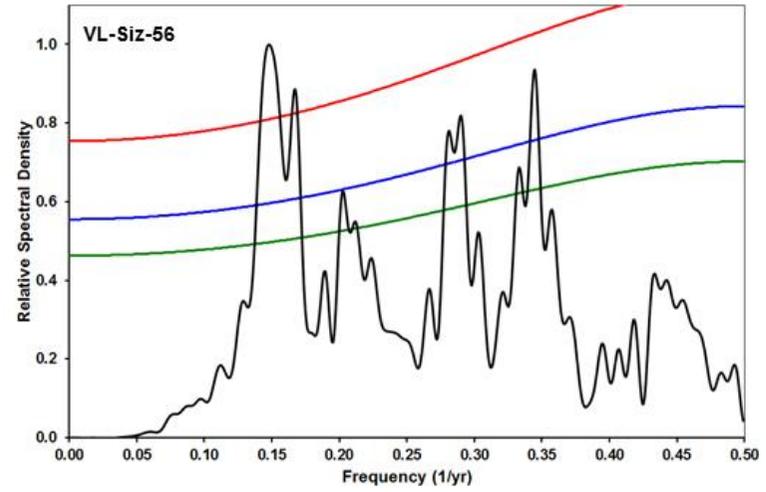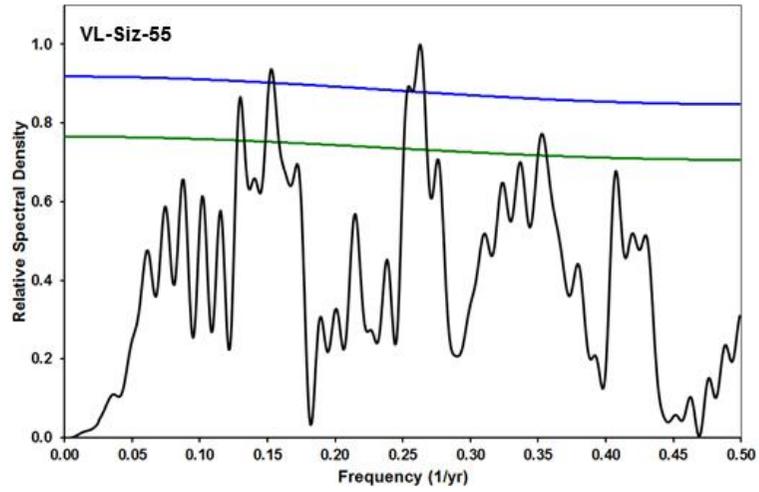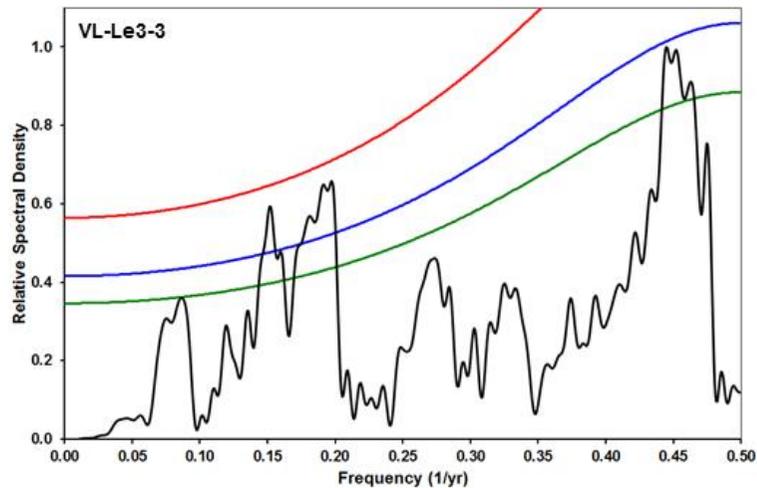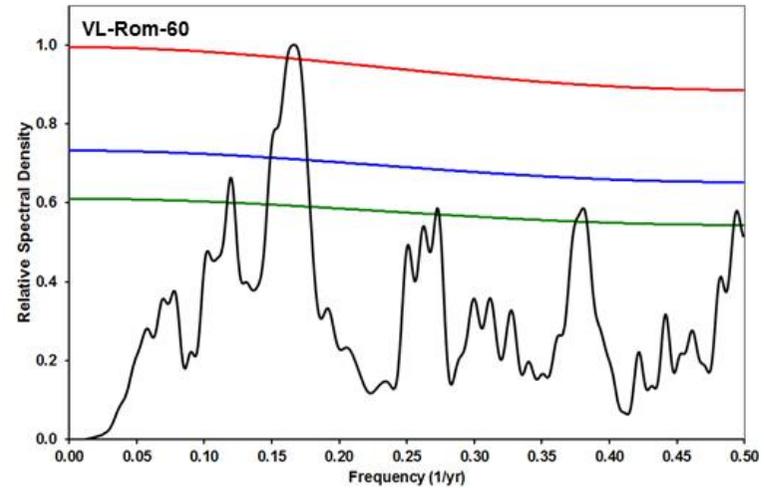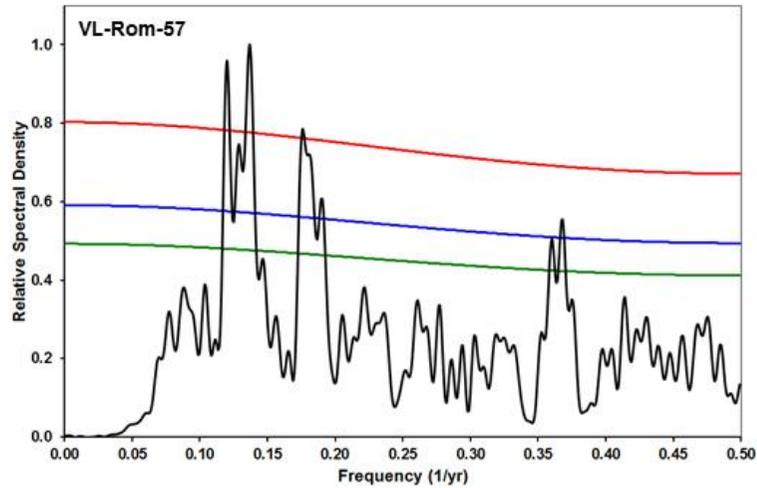
Appendix 2: Frequency Analysis by means of Multi-Taper Method based on the original times-series (kSpectra version 3.5). Number of components was chosen as 10. Window sized varied between 1/2 and 1/3 of time-series length. Window size VL-Siz-14: 60, VL-Siz-33: 70, VL-Siz-35: 30, VL-Siz-46: 40, VL-Siz-55: 40, VL-Siz-56: 50, VL-Siz-58: 30, VL-Siz-60: 60, VL-Rom- 57: 70, VL-Rom-60: 40 and VL-L3-3: 45. Confidence Intervals: red (99%), blue (95%) and green (90%)