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OXYGEN STABLE ISOTOPE ANALYSES ON *AMEGHINOMYA ANTIQUA* SHELLS: A PROMISING TOOL FOR PALAEOENVIRONMENTAL RECONSTRUCTION ALONG THE QUATERNARY PATAGONIAN ARGENTINA COAST?

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ABSTRACT: There are only a few data concerning the Quaternary climate fluctuations in the marine environments of the Atlantic Patagonian coast. In this regard, the aragonitic shell of the *Ameghinomya antiqua* bivalve offers the possibility to study the climate variability and the seasonal cycles of sea water temperature in the region at different geological times. We compared oxygen isotopic profiles along the shell-growth axis on four well-preserved *A. antiqua* bivalves collected from marine coastal deposits of Marine Isotope Stage (MIS) 7, MIS 5, Holocene (7.284 ±140 yr cal BP), and from the present-day active beach of Bahía Bustamante (Patagonia Argentina). Shell ontogeny was determined through the annual growth lines recorded along the external region of the shell, and was also verified by cross-section analyses. The fossil bivalves were around 15 years old, while the Present day shell was 10 years old. When the modern climatic data available are compared, the higher $\delta^{18}O_{shell}$ values represent the cold season, while the lower $\delta^{18}O_{shell}$ values indicate the warm season. The $\delta^{18}O_{shell}$ ranges indicate different environmental conditions and seasonal temperature variations between specimens. By assuming, a constant $\delta^{18}O_{w}$ calculated with the limited environmental data available, superficial seawater temperatures are estimated from the specimens. These paleotemperatures may overestimate water temperatures, and shell formation seems to occur with an offset from expected oxygen isotopic equilibrium with the water in which they lived. Meanwhile, *A. antiqua* shells are suitable bioarchives for the comparison of seasonal patterns throughout the Quaternary, thus constituting another proxy for the evaluation of paleoclimatic and paleoenvironmental changes in the Patagonia region.

Keywords: Oxygen isotopes, bivalve shells, *Ameghinomya antiqua*, Quaternary, seasonality, Patagonia.

1. INTRODUCTION

It is well known that mollusk shells can preserve important data on the biology of the organisms and on the environmental conditions present during their growth (Jones & Gould, 1999; Richardson, 2001; Goodwin et al., 2010; among others). Growth rate and time are controlled by many factors, including temperature (Jones et al., 1989; Schöne et al., 2002), salinity (Koike, 1980), age (Jones et al., 1989), reproductive cycle (Sato, 1995), tidal cycles, intertidal positions (Ohno, 1989; Goodwin et al., 2001) and nutrient availability (Schöne et al., 2003; Goodwin et al., 2010). However, temperature seems to be the main factor controlling the intra-annual growth rate in most of the species (Koike, 1980; Goodwin et al., 2001; Schöne et al., 2002).

The oxygen and carbon stable isotope composition of marine mollusk shells has proven to be a valuable proxy for reconstruction of the environmental history and climatic conditions during the Quaternary (Epstein et al., 1953; Rhoads & Lutz, 1980; Brand et al., 1987; Aguirre et al., 2002; Jones et al., 2005; Schöne et al., 2004, 2005; Watanabe et al., 2004; Carré et al., 2005; Miyaji et al., 2010; Schöne & Gillikin, 2013; Gordillo et al., 2015; among others). In particular, variations in oxygen and carbon isotope ratios within the shell carbonate of mollusks have been used to track present and past annual marine temperature cycles (Jones, 1983; Jones et al., 1989; Jones & Allmon, 1995). This data can be used to assess seasonal changes (Jones & Allmon, 1995) and to infer productivity and marine salinity fluctuations (Geary et al., 1989; Goodwin et al., 2009) The isotopic profiles of marine mollusks thus have great potential for high resolution paleoenvironmental reconstruction. Annual seasonal cycles in oxygen and carbon isotopes also provide an independent method for ontogenic age determination of mollusks whose life span and growth rates may be unknown (Jones & Allmon, 1995).

Patagonian raised coastal deposits contain rich mollusk faunal assemblages, which are potentially useful for the detailed paleoclimatic reconstruction of periods of sea-level highstand as far back as MIS 11

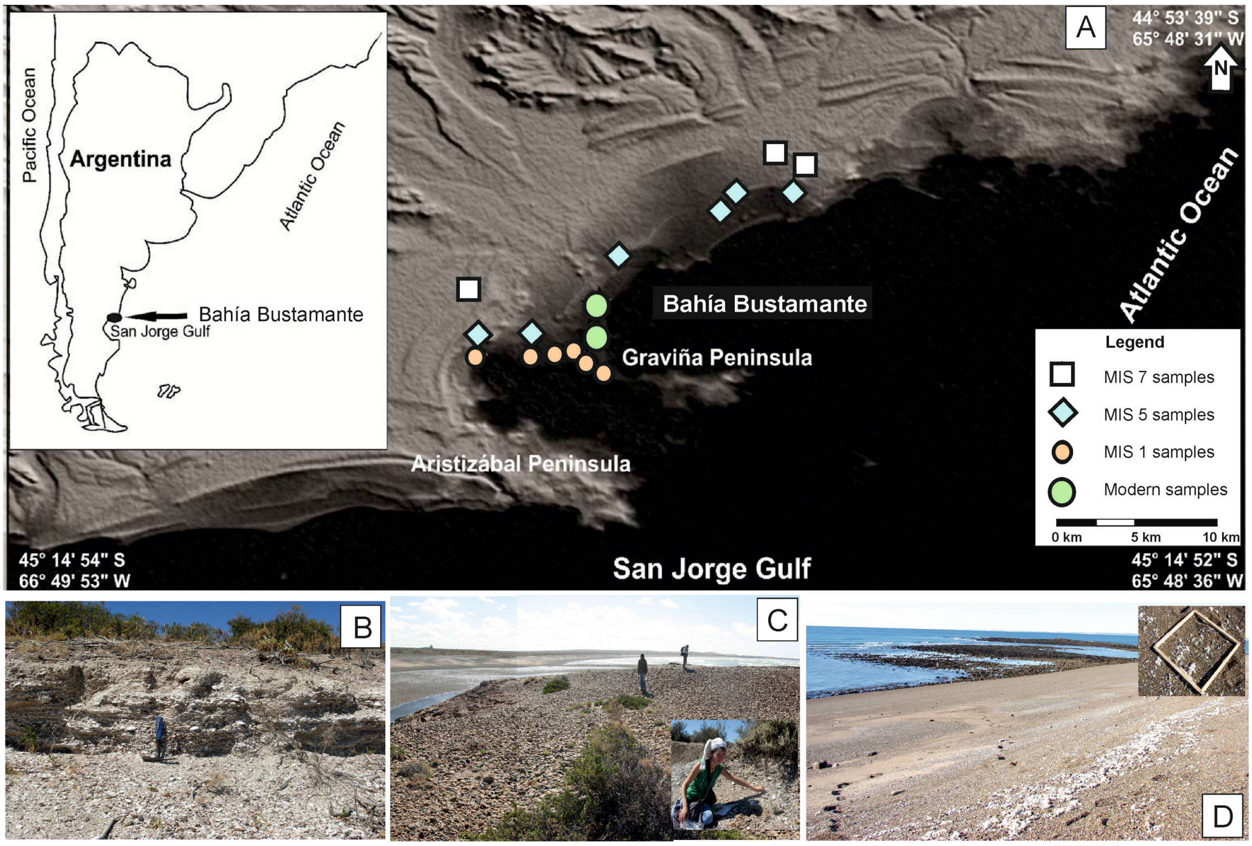

Fig. 1 - Modified from Boretto et al. (2014). A. Location of the study area. B. Pleistocene (MIS 5) deposits. C. Holocene (7.284±140 yr BP) deposit. D. Present-day shell accumulation on an active beach.

(Schellmann & Radtke, 2000, 2007, 2010; Pappalardo et al., 2015; Zanchetta et al., 2012, 2014; Bini et al., 2018). The shells of the aragonitic bivalve *Ameghinomya antiqua* are particularly suitable to investigate the environmental conditions of this region during the Quaternary, owing to the excellent preservation of the fossil shells and to their suitability for geometric morphometric analysis (Boretto, 2013; Boretto et al., 2014).

In this paper, conceived as a pilot study, we discuss oxygen isotopic composition along the external shell growth axis of fossil and modern *A. antiqua* shells collected at Bahía Bustamante in Patagonia, Argentina (Fig. 1), including the Present day active beach as well as the deposits from the Holocene and Marine Isotope Stages (MIS) 5 and 7. The aim of this work is to establish whether oxygen stable isotope analyses on *A. antiqua* shells can indicate local environmental conditions in the different interglacial periods and, if this is the case, to evaluate the seasonal conditions during different interglacial periods.

2. GEOGRAPHICAL AND GEOLOGICAL SETTINGS

The geology of Bahía Bustamante (45°13'-44°54'S and 66°50'-65°48'W; San Jorge Gulf, Argentina; Fig. 1) is dominated by Quaternary marine coastal deposits, organized in a succession of beach ridges and marine terraces composed of accumulated sand, gravel and mollusk shells (Feruglio, 1950; Cionchi, 1987,1988; Rutter et. al., 1989, 1990; Schellmann & Radtke, 2000, 2007, 2010; Aguirre et al., 2005; Ardolino et al., 2008; Isola et al., 2011; Boretto, 2013; Boretto et al., 2014). These deposits, which extend mostly parallel to the current coastline, were dated by Schellmann & Radtke (2000, 2010) through electron spin resonance and α-spectrometric ^{230}Th/^{234}U methods at MIS 7 (15-23 m a.s.l.) and MIS 5 (10-19 m a.s.l.), and through radiocarbon dating for the Holocene (3-10 m a.s.l.). Past landforms and deposits are particularly well-preserved because weathering processes are very limited (Isola et al., 2011) as a result of the arid environment of the area during the Quaternary. These deposits mostly overlie the Marifil Complex, which consists of Jurassic rhyolites, ignimbrites and volcanoclastic conglomerates (Sciutto et al., 2000; Lema et al., 2001; Isola et al., 2011).

The local climate is characterized by two main meteorological features: constant dry winds blowing strongly from the west, and low precipitation (<300 mm/yr) (Coronato et al., 2008). The moderate thermal amplitude allows the growth of a sparse cover of grass and shrubby vegetation, consistent with the semiarid climate of Patagonia. Wave action and sea level oscillations are the main morphoclimatic agents responsible for the past and present landscapes. A high-energy system with intense storms and a macrotidal (>4 m) regime dominate the area (Isla & Bujaleswsky, 2008), thus representing the main morphodynamic agents for shaping of the coastal zone.

3. BIVALVE *AMEGHINOMYA ANTIQUA*

Ecology. This species is a benthic bivalve typical of the Magellanic region (Balech & Ehrlich, 2008), which extends along the Atlantic Ocean from 34° S to the Beagle Channel (54° S) and along the Pacific Ocean as far as Callao (12°77' S) in Peru (Castellanos, 1967; Márquez et al., 2010). It lives in the sandy soft-bottom substrate, from the shallow intertidal up to 100 m depth in temperate-cold waters (Castellanos, 1967). In the Patagonian area the maximum depth of this species is 60 m, and its absence at greater depths is probably associated with an increase in the fine-sediment fraction (Zaixso, 1996).

Shell morphology. The *A. antiqua* shells have a typically equivalve external aspect, with a subcircular contour, prosogyrous and sub-central umbos and a strong external ligament. The external sculpture consists of fine, sometimes high, concentric lines, crossed by numerous radial grooves that are wide and flattened, giving it a general cross-linked appearance (Escati Peñaloza, 2005; Escati Peñaloza et al., 2010; Zaixso & Boraso, 2015).

Individual growth. The adult stage of bivalves is related to the size achieved by the first sexual maturity (Calvo et al., 1998). *A. antiqua* reaches reproductive maturity at around 2 years of age, with a length of around 13-22 mm. The reproductive processes occurring in the austral winter months and the effort necessary to decrease the energy invested in growth (Escati Peñaloza, 2005) generate seasonal growth patterns, which cause marked annual growth rings in the winter (Verdinelli et al., 1976, Clasing et al., 1994). Shell growth takes place during the warm spring-summer season, so that light or opaque calcium carbonate bands are deposited. Dark or translucent increments are generated during the autumn-winter months, and a conspicuous mark is produced on the outer region of the shell before the end of the cold season (Fig. 2) (Escati Peñaloza, 2005; Escati Peñaloza et al., 2010). The number of growth rings observed on the outer shell accumulated per year and their formation time have been studied for *A. antiqua* through: (1) analysis of the correspondence between internal microlines (cross sections) and external rings (Andaur, 1999); (2) mark-recapture experiments (Clasing et al., 1994); (3) monitoring the edge of the shell during the growing season (Reyes et al., 1992); and (4) other methods of age determination and growth parameter estimation (Urban, 1994, 1996; Escati Peñaloza, 2005; Escati Peñaloza et al., 2010). The results of these studies clearly indicate that the use of external growth rings observed in the outer shell region is suitable to correctly determine the ontogeny and to estimate the growth rate of this species (Escati Peñaloza, 2005).

4. MATERIAL AND METHODS

4.1 Field sampling

Adult specimens of *A. antiqua* were collected in the area of Bahía Bustamante (Fig. 1). Fossil specimens from Holocene and Pleistocene beach ridges were taken from randomly chosen bulk samples (50 cm^3) during field trips in January-February 2011. Radiocarbon dating of the Holocene beach ridge yielded an age of 6758 yrs BP (Schellmann & Radtke, 2000, 2010) with a calibrated age of 7.284 ±140 yrs cal BP (Reimer et al., 2013). The sampled MIS 5 and MIS 7 deposits were dated with electron spin resonance (ESR) by Schellmann & Radtke (2000), and were assigned ages of 111 ±19 ka and 220 ±22 ka, respectively. For comparison with fossil clam data, Present day shells were randomly sampled by means of a 1x1 m quadrant (Fig. 1D) from the present active beach. These modern samples had preserved hinges and remains of organic matter, which indicated that their death had taken place only a short time before collection.

4.2 Preparation of the *A. antiqua* samples in the laboratory

A total of four shells, one for each age from the Present day (active beach), Holocene, MIS 5 and MIS 7 deposits, were selected for this study (Table 1). The bivalves chosen had well-preserved paired valves to avoid individual shells, which could have experienced postmortem transport, thus minimizing any erosion and reworked material from older units. They were whole and well-preserved; the growth rings on the external shell region were clearly discernable with the naked eye. The shells were chosen arbitrarily and were ultrasonically cleaned in deionized water for 10 minutes, so as to

Shell samples collected from coastal deposits*	Min $\delta^{18}O$ [‰]	Max $\delta^{18}O$ [‰]	Mean $\delta^{18}O$ [‰]	SD	$\delta^{18}O$ Range [‰]	Seasonal temperature variation [°C]	Ontogeny/ external rings [years]	Max Length [mm]
MIS 7 ~220 ±22 ka***	- 0.96	1.56	0.36	0.50	2.52	11	15	50
MIS 5 ~111 ±19 ka***	- 0.98	1.97	0.55	0.53	2.95	13	14	58
Holocene 7.284 ±140 BP**	- 0.67	1.53	0.43	0.52	2.2	9.5	15	50
Present day	- 1.47	1.57	0.30	0.68	3.04	13.2	10	38

Tab. 1 - List of *Ameghinomya antiqua* specimens collected for oxygen isotopes. The sampled sites were defined stratigraphically and dated by *Schellmann & Radtke (2000): **ESR method, ***^{14}C method. The Pleistocene samples are correlated with the respective Marine Isotopic Stage (MIS).

remove any sediment and organic matter and were then dried in an oven at 60°C. X-ray diffraction analyses of powder from specimens indicated that the shell material was virtually pure aragonitic (100% aragonite) (Consoloni, 2013).

4.2.1 Ontogenic age of each sample

The individual age of each specimen was calculated using the method employed by Escati Peñaloza (2005), which consists in recording the number of growth rings that have developed along the growth axis of each shell (Fig. 2). To facilitate the identification of the rings, the outer shell surfaces were polished to show the reticular sculpture and to expose the clear growth marks. Escati Peñaloza (2005) considered these annual bands to be well-defined, sharply delineated and dark in color, forming conspicuous, easily identifiable concentric rings. However, cross-sections of the growth lines were also taken to ensure accuracy. Each selected shell was externally strengthened with a layer of epoxy resin to protect it from breakage. The shells were then cross-sectioned (perpendicular to the growth lines) by using a low-speed precision saw (Phoenix Alpha, Buehler), and were polished by using a manual grinder-polisher (Buehler) and sand paper with the three different grain sizes 15, 10 and 5 µm. One unstained cross-section was employed for growth pattern analysis, and digital images were taken through a Leica S6D Greenough stereo microscope with a 6.3:1 zoom and integrated video/photo port equipped with sectoral dark field illumination. Ontogenetic age was determined by counting the annual growth increments under a stereo microscope (Leica S6D).

4.2.2 Isotopic analyses of shells

For stable oxygen isotope analyses of shell carbonate ($\delta^{18}O_{shell}$), a total of 206 samples were taken between the umbo and the external ventral margin (area of latest growth) in order to obtain calcium carbonate powder. A Dremel microdrill and a 0.5 mm bit were employed to drill the external shells in ontogenetic sequence (Fig. 2). Each powdered $CaCO_3$ sample weighed approximately 0.15 mg. After each new drilling, the tools and the shell were cleaned by using compressed air to prevent contamination. The consecutive drill holes were spaced approximately 1 mm apart to achieve a high resolution and a continuous seasonal record. This drilling method on the external region of the shell was used on mollusks by different authors, such as Killingley (1981), Jones (1983), Krantz et al. (1984), Jones & Allmon (1995), Bojar et al. (2004), Yan et al. (2009), Jolivet et al. (2015), Peharda et al. (2017), among others. Although other authors suggest that shell cross-section drilling is more suitable (Jones et al., 1989; Goodwin et al., 2001; Schöne et al., 2002, 2003, 2004; Yan et al. 2012), we believe that the methodology applied for the purpose of this study is appropriate since the ontogeny is calculated on the basis of the external growth rings correlating with the internal microlines analyzed in cross-sections by other authors (Clasing et al., 1994; Andaur, 1999; Escati Peñaloza, 2005). Stable isotope analysis was carried out using a Gas Bench II (Thermo Scientific) coupled to a Delta XP IRMS (Finnigan) at the Institute of Geosciences and Earth Resources of the Italian National Research Council (IGG -CNR) in Pisa (Italy). Carbonate samples of ca. 0.15 mg of $CaCO_3$ were dissolved in H_3PO_4 (100%) for an hour at 70°C. All the results reported are relative to the V-PDB International Standard. Sample results were corrected by using the International Standard NBS-18 and a set of 3 internal standards, previously calibrated according to the international standards NBS-18 and NBS-19 (Negri et al., 2015). Analytical uncertainty for $\delta^{18}O$ was 0.13‰. Owing to the sampling difficulties in the marginal sector of the shell, the last few years of the specimens were not recorded in the isotopic data.

4.3 Environmental variables: sea surface temperature (SST), salinity (SSS), isotopic water composition ($\delta^{18}O_w$)

According to the period of collection and annual counting, the Present day specimen lived between the years 2000 and 2010. Monthly records of sea surface temperature (SST) in Bahía Bustamante were obtained from NASA Earth Data by the Moderate Resolution Imaging Spectroradiometer (MODIS) (http://giovanni.gsfc.nasa.gov) (Fig. 6). Direct *in situ* measurement was not available, so we compared the recorded SST with information accessed from a marine station near the study area belonging to the Argentine Naval Hydrography Service (http://www.hidro.gov.ar/ceado/Ef/Inventar.asp). Sea surface salinity (SSS) data for the specific study area could not be accessed from the MODIS satellite, but values related to this environmental variable were obtained from previous studies in the region. Fernández et al. (2007) measured an SSS value of 33,362 PSUs during the autumn-winter season and Louge et al. (2004) recorded an SSS value of 33,766 PSUs for the summer season.

In shallow water environments, SST, SSS and isotopic water composition ($\delta^{18}O_w$) can undergo seasonal and inter-annual variations (e.g. Yan et al., 2012). The only $\delta^{18}O_w$ data available in Bahía Bustamante are provided by Consoloni (2013), who reported a summer (February 2011) value of -0.38‰. The annual mean $\delta^{18}O_w$ value (-0.50‰) was taken from the Global Seawater Oxygen-18 Database (http://data.giss.nasa.gov/o18data/) and calculated from the gridded data, using salinity and PO_4^* to infer $\delta^{18}O_w$ values. More recently, Rubo et al. (2018) measured $\delta^{18}O_w$ and salinity (PSU) along different places located to the south of the study area in Patagonia, taking into account this database (https://data.mendeley.com/datasets/sz2v8ztb38/1) and the values measured by Meredith et al. (1999) for the Atlantic Ocean. The $\delta^{18}O_w$ for the region was reconstructed by applying the method proposed by Colonese et al. (2011, 2012) and Yan et al. (2012) (see Section 4.4).

4.4 Paleotemperatures

The $\delta^{18}O_{shell}$ value mostly depends on changes in temperature and in the oxygen isotope composition of the ambient water ($\delta^{18}O_w$) (e.g. Colonese et al., 2011, 2012; Yan et al., 2012). If the $\delta^{18}O_w$ value is known and

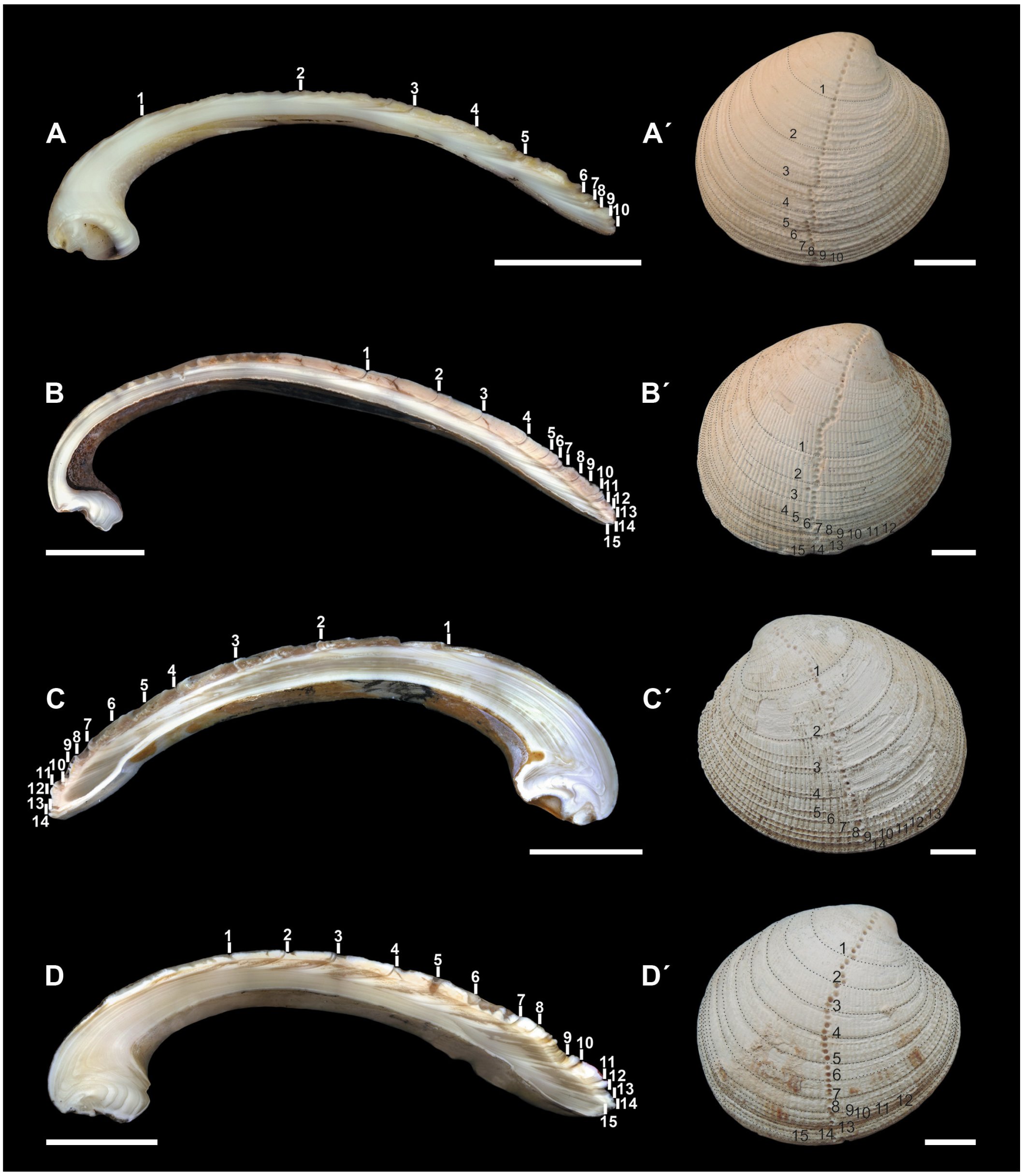

Fig. 2 - Shells sampled along the growth axis for isotopic analyses. Scale bar =10 mm. A, A': Present day specimen; B, B': Holocene specimen; C, C': MIS 5 specimen; D, D': MIS 7 specimen.

if the shells were formed in oxygen isotopic equilibrium (or near) with the water in which they lived, then the water temperature can be reliably reconstructed from $\delta^{18}O_{shell}$ (Epstein et al., 1953).

For reconstruction of the water temperatures from $\delta^{18}O_{shell}$ values ($T_{\delta 18O}$) of *A. antiqua*, we used the Grossman & Ku (1986) paleothermometry equation with a PDB to V-SMOW scale correction of -0.27‰ modified by Dettman et al. (1999). The corrected equation is:

$$T_{\delta 18O}\ (°C) = 20.34 - 4.34\ [\delta^{18}O_{shell} - (\delta^{18}O_{water} - 0.27)] \quad (1)$$

In shallow water environments, salinity and, hence, $\delta^{18}O_w$ may underlie seasonal and interannual variations that can hamper precise temperature estimates based on $\delta^{18}O_{shell}$ values, unless the $\delta^{18}O_w$ value is closely monitored. Since no long-term observations were available for the study area, the $\delta^{18}O_w$ signature was calculated from sea surface salinity (SSS), considering the annual average of the 33.564 PSU value and of the $\delta^{18}O_w$ values measured along the region. With this aim, a local freshwater mixing line was constructed for this area, based on the dataset of the NASA Goddard Institute for Space Studies (GISS) (Meredith, 1999; Schmidt, 1999; Bigg & Rohling, 2000) and on the data provided by Consoloni (2013), Yan et al. (2012), M. Sol Bayer

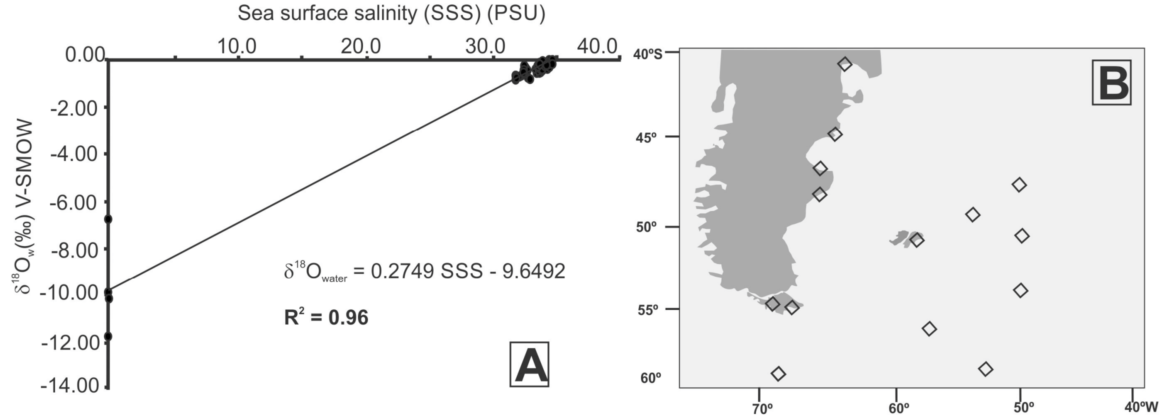

Fig. 3 - A. Freshwater mixing line constructed from regional $\delta^{18}O_{water}$ and sea surface salinity (SSS) data (Appendix). B. Region from which the aforementioned data derives.

(pers. comm to GB., 2016) and Rubo et al. (2018). The respective equation is as follows (Fig. 3). (Appendix):

$$\delta^{18}O_w \text{‰} = 0.2749 * SSS - 9.6492 \qquad (2)$$

To compute water paleotemperatures from $\delta^{18}O_{shell}$ ($T_{\delta 18O}$), we assumed a $\delta^{18}O_w$ value of - 0.42‰ based on long-term average salinity (see Section 4.3) and Eq. (2). Therefore, this value is considered for the calculation of paleotemperatures for the Present day and Holocene (7,284 BP) specimens, assuming that no significant changes in the isotopic composition of sea water occurred in the last 7.000 yrs, when global sea level was mainly stable (Lambeck et al., 2014). By comparison, the annual mean $\delta^{18}O_w$ value of -0.50‰ was also used to estimate temperature on the Present day sample. As the $\delta^{18}O_w$ composition for the MIS 5 and MIS 7 for the study area was unknown, we considered the $\delta^{18}O_w$ re-

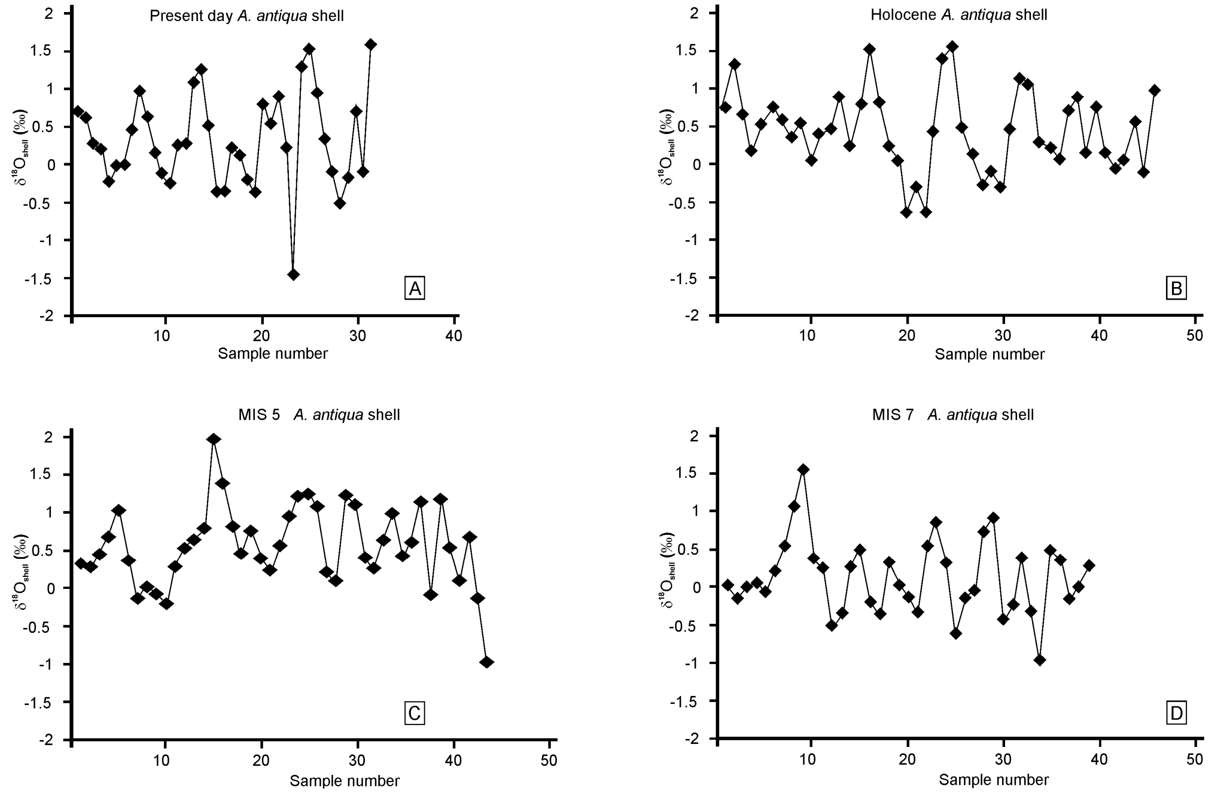

Fig. 4 - Oxygen isotopic profiles measured on *A. antiqua* shells. A. Present day specimen. B. Holocene specimen. C. MIS 5 specimen. D. MIS 7 specimen.

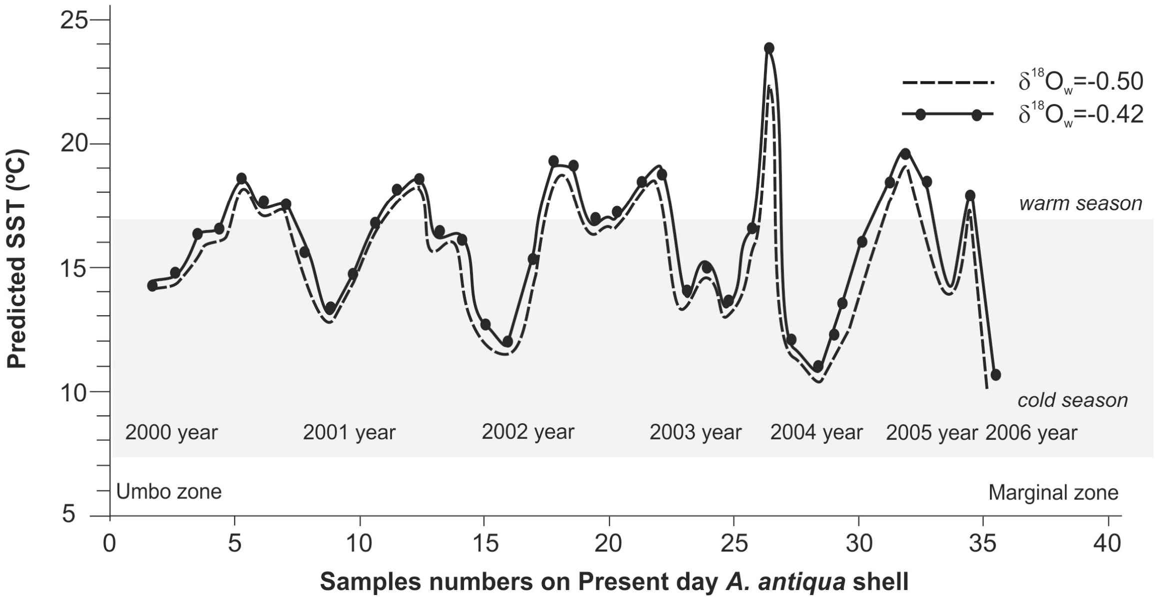

Fig. 5 - Predicted SSTs from the $\delta^{18}O_{shell}$ of the Present day *A. antiqua* specimen. SSTs were computed by using $\delta^{18}O_w$ of -0.50‰ (mean value from the Global Seawater Oxygen-18 Database) and the $\delta^{18}O_w$ values of -0.42‰ obtained from Eq. (2) for the region. Estimated SSTs fell into the range of measured SSTs (grey band), and the maximum SST was highest in one exceptional year.

constructed for the global ocean by Shakun et al. (2015). According to Shakun et al. (2015) average values of the MIS5 was of $\delta^{18}O_w$ of -0.45‰ and for the MIS 7 was of -0.31‰ V-SMOW. The MIS 5-MIS 7 paleotemperature values should therefore be treated with caution, since no precise data can be provided for regional changes in salinity for the different interglacial periods.

5. RESULTS

5.1 Present day shell

This specimen lived until January 2011 and the ontogenetic study indicates an age of 10 years (Fig. 2). The shell length is 38 mm, and the first year represents 26% of the total, with 64% of growth concentrated during the first 3 years. Intra-annual $\delta^{18}O_{shell}$ presents clear seasonal fluctuations (Table 2), with positive +0.69‰ and negative -0.30‰ average values associated with the colder and warmer months. The maximum value is +1.57‰ and the minimum is -1.47‰; the $\delta^{18}O_{shell}$ annual mean value is 0.31‰, while $\Delta\delta^{18}O_{shell}$=3.04‰, and there is a seasonal average temperature spread of ca. 13.2°C (Fig. 4A).

The reconstructed SSTs for Present day *A. antiqua* were calculated by using both the $\delta^{18}O_w$ of -0.42‰ for the region and the average value $\delta^{18}O_w$ of -0.50‰ obtained from the Global Seawater Oxygen-18 Database. The results (Fig. 5) broadly agree with the mean interannual data recorded. The SSTs predicted present some offset with respect to the measured SST available for the area. For instance, the warmest value (more than 23°C) estimated for spring-summer 2004 is higher than the maximum temperature recorded for that year.

The relationship between the $\delta^{18}O_{shell}$ data, the

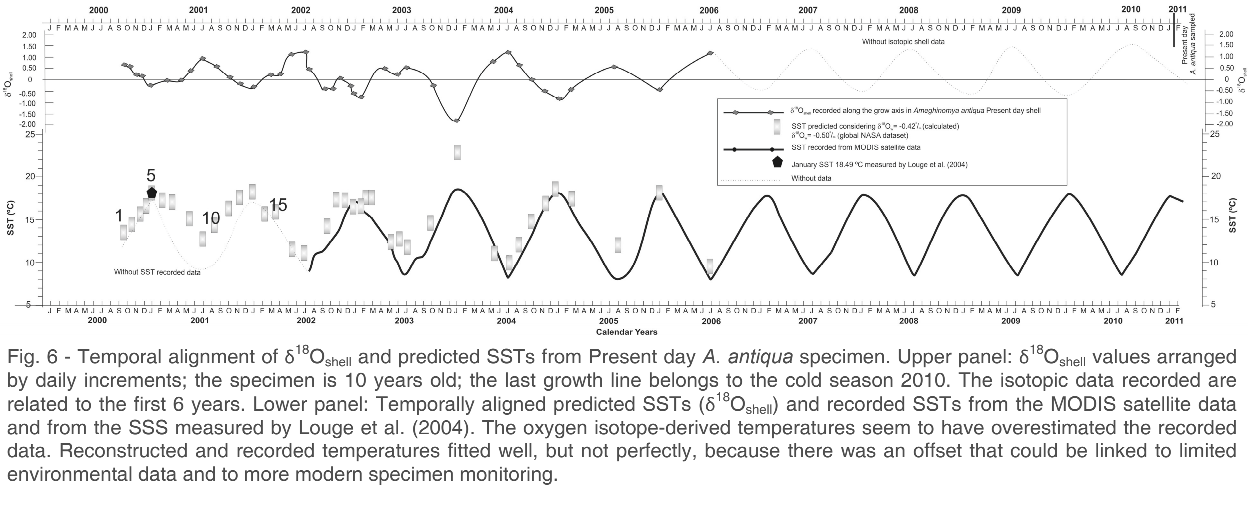

Fig. 6 - Temporal alignment of $\delta^{18}O_{shell}$ and predicted SSTs from Present day *A. antiqua* specimen. Upper panel: $\delta^{18}O_{shell}$ values arranged by daily increments; the specimen is 10 years old; the last growth line belongs to the cold season 2010. The isotopic data recorded are related to the first 6 years. Lower panel: Temporally aligned predicted SSTs ($\delta^{18}O_{shell}$) and recorded SSTs from the MODIS satellite data and from the SSS measured by Louge et al. (2004). The oxygen isotope-derived temperatures seem to have overestimated the recorded data. Reconstructed and recorded temperatures fitted well, but not perfectly, because there was an offset that could be linked to limited environmental data and to more modern specimen monitoring.

recorded SST in the study area during the specimen's lifetime and the predicted SST is shown in Figure 6. According to this alignment, the annual growth increments were formed between the spring-summer season of the following year, and the annual minimum and maximum temperatures were recorded in July-August and January, respectively. The $\delta^{18}O_{shell}$ profile (counting the positive peaks) shows six cycles, which seems to represent six years. On the basis of this observation we can conclude that annual growth lines (shell portion representing the time interval of strongly reduced or even halted shell growth) were deposited between the cold seasons (autumn-winter) (Fig. 6).

The predicted inter-annual mean SST was 16°C, while the SST recorded by the MODIS satellite was 12.5°C, thus presenting an average offset of 3.5°C. $T_{\delta18O}$ was overestimated with respect to the SST, corresponding to an average $\delta^{18}O_{shell}$ of -0.80‰, with a minimum of 11°C SST predicted from the shell and a maximum of 24°C. The maximum and minimum average temperatures recorded by MODIS were 17°C and 8°C respectively, and the monthly mean SST ranged from 16°C in summer (Dec-Jan-Feb-Mar) to 9°C in winter (Jun-Jul-Ag-Sep), with an intra-annual variability of around 7°C. Seasonal periodicity was observed in the isotopic profile of the valve, and therefore was also present in the predicted SSTs of this data, associated with the environmental data recorded in Bahía Bustamante. With respect to the offset, it should be noted that there was little discrepancy between the predicted and the recorded data, and that cyclicality in the isotopic record was maintained throughout the lifetime of the specimen. These preliminary results indicated that $\delta^{18}O_{shell}$ can record seasonal changes in SSTs and in $\delta^{18}O_w$. Some offset with measured data are present, but this can also related to the poor record of the environmental data. It would be interesting to deepen and confirm these preliminary studies with more data obtained from the Patagonian coast and through appropriate data monitoring.

With this analysis in mind, we considered this species useful for reconstructing seasonal patterns in Quaternary coastal sites.

5.2 *A. antiqua* fossil specimens

The Holocene specimen lived for 15 years (Fig. 2). The shell length was 50 mm. The first and second years represented 50% of the increments; from the third to the fifth years, this percentage was 21%, and then the increments decreased considerably. This sample had $\delta^{18}O_{shell}$ values that indicated a strong seasonality (Fig. 4B). The extreme values were -0.67‰ and 1.53‰ (Table 2), which were associated with the warmest and coldest seasons respectively, while a mean inter-annual (i.e. averaged over all years) value was 0.43‰. The warm season (WS) mean value was -0.18‰, and the cold season (CS) mean value was 1.03‰. It was possible to distinguish 4 well-marked positive peaks (1.31, 1.53, 1.50, 1.12‰) and 3 clearly negative points (-0.67, -0.64, -0.31‰), with the $\Delta\delta^{18}O$ at 2.2‰, and the seasonal temperature variation at ca. 9.5°C (assuming no changes in sea water isotopic composition). If we consider the $\delta^{18}O_w$ value of -0.42‰ over time, we could apply Eq. (2) to estimate SSTs from this specimen. In this case, the predicted annual mean SST was 15°C, with a maximum of 20°C and a minimum of 11°C (Fig. 7).

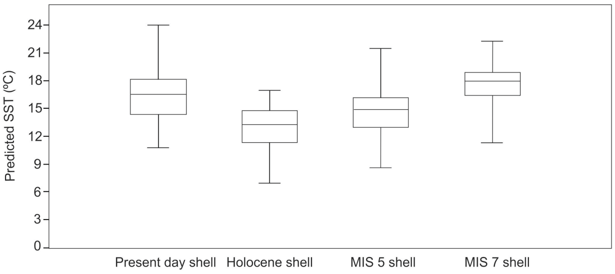

Fig. 7 - Predicted SSTs from the *A. antiqua* specimens through Eq. (2), taking into account the estimated $\delta^{18}O_w$ for each time interval in the region.

The MIS 5 sample lived for 14 years (Fig. 2). The shell length was 58 mm, and the first to the fourth years of life produced 73% of the increments (with 37% corresponding to the first year). In the isotopic profiles recorded for this specimen, the minimum $\delta^{18}O_{shell}$ value was -0.98‰ and the maximum was 1.97‰ (Table 2), with an annual mean value of 0.55‰. Overall, the $\delta^{18}O_{shell}$ curve behaviour tended to be positive, with no prominent peaks, and was recorded for 8 years (Fig. 4C). The $\delta^{18}O_{shell}$ WS mean value was 0.02‰, and the CS mean value was 1.13‰; the $\Delta\delta^{18}O_{shell}$ was 2.95‰ and the estimated seasonal temperature variation was 13°C.

The number of growth rings in the MIS 7 specimen indicated an age of 15 years (Fig. 2), and the shell length was 50 mm. As in the other samples, the increments developed significantly during the first four years of life (ca. 74%). The $\delta^{18}O_{shell}$ values for the MIS 7 specimen (Fig. 4D) ranged from -0.96‰ to 1.56‰, and the annual mean value was 0.13‰ (Table 2). The WS mean value was -0.36‰, while the CS mean value was 0.78‰. In the oxygen isotope curve, 3 peaks stood out as well-marked, with values higher than > 0.50‰ (1.56, 0.86, 0.92‰). These would suggest a further temperature decrease in the cold season during these years, taking into consideration the average annual value. The $\Delta\delta^{18}O$ was 2.52‰, and the average seasonal temperature variation recorded in this sample was ca. 11°C.

The paleotemperatures reconstructed from Pleistocene specimens were affected by a lack of detailed estimations of the isotopic composition of the seawater. In this sense, Shakun et al, (2015) provided a dataset of $\delta^{18}O_w$ for the global ocean during the 800 kyr glacial-interglacial cycles through different records. This data could be improved with more geographical datapoints and variable measurements. According to Shakun et al. (2015) $\delta^{18}O_w$ values the annual predicted mean SST ($\delta^{18}O_w$=-0.45‰) to be 15°C, the maximum recorded 22° C and the minimum 9°C for the MIS 5 specimen. The predicted values for the MIS 7 sample were: an annual predicted mean SST ($\delta^{18}O_w$=-0.31‰) of 17°C, a maximum of 22°C and a minimum of around 11°C.

Number of sample along growth axis shell	$\delta^{18}O_{shell}$ ‰ (V-PDB)			
	Present day Shell	**Holocene shell**	**MIS 5 shell**	**MIS 7 shell**
1	0,69	0,73	0,33	0,03
2	0,61	1,31	0,27	-0,14
3	0,27	0,65	0,45	0,01
4	0,19	0,17	0,68	0,06
5	-0,23	0,52	1,03	-0,05
6	-0,02	0,74	0,37	0,22
7	-0,01	0,59	-0,14	0,55
8	0,44	0,37	0,01	1,08
9	0,97	0,54	-0,08	1,56
10	0,62	0,09	-0,21	0,39
11	0,15	0,41	0,28	0,26
12	-0,13	0,47	0,53	-0,51
13	-0,26	0,9	0,64	-0,34
14	0,25	0,25	0,79	0,27
15	0,27	0,79	1,97	0,48
16	1,08	1,53	1,38	-0,2
17	1,25	0,81	0,82	-0,35
18	0,5	0,22	0,47	0,33
19	-0,37	0,05	0,76	0,03
20	-0,36	-0,67	0,4	-0,13
21	0,13	-0,27	0,24	-0,32
22	-1,47	-0,64	0,56	0,55
23	-0,21	0,42	0,95	0,86
24	-0,37	1,38	1,21	0,33
25	0,79	1,5	1,24	-0,6
26	0,53	0,48	1,08	-0,14
27	0,89	0,14	0,21	-0,03
28	0,22	-0,29	0,11	0,74
29	0,11	-0,1	1,22	0,92
30	1,29	-0,31	1,11	-0,42
31	1,52	0,46	0,41	-0,23
32	0,94	1,12	0,26	0,39
33	0,33	1,04	0,64	-0,32
34	-0,11	0,28	0,99	-0,96
35	-0,52	0,2	0,43	0,5
36	-0,18	0,07	0,61	0,36
37	0,7	0,69	1,13	-0,15
38	-0,1	0,87	-0,08	0,01
39	1,57	0,15	1,18	0,29
40		0,76	0,54	0,03
41		0,15	0,1	
42		-0,07	0,67	
43		0,04	-0,13	
44		0,55	-0,98	
45		-0,12		
46		0,98		

Tab. 2 - Sequential $\delta^{18}O_{shell}$ values of Present day *A. antiqua* specimen and fossil shells from Bahía Bustamante.

6. DISCUSSION

According to these preliminary results, *A. antiqua* shells from Patagonia should be suitable to the use of oxygen isotopes studies for evaluating marine environmental changes in the Argentine Patagonian region during the middle-late Quaternary. Our analyses indicate that *A. antiqua* has annual growth rates that ensure sufficient seasonal isotopic resolution. It is interesting to note the decreasing growth rate after 3-4 years in the four specimens, hence, the annual rings accumulate in the outer marginal zone of the shell. As discussed by Schöne (2008), mollusk shells record the environment only during growth; therefore, growth lines indicate a slowdown or complete cessation of shell production. Accordingly, the oxygen isotope values in the outer part of the shell would not be as reliable as in the rest of the valve. Data show that *A. antiqua* grows faster during summer and at a slow rate during winter, and it records a seasonal temperature amplitude recorded in the $\delta^{18}O_{shell}$. In this respect, Rubo et al. (2018) suggested that the isotope-based climate reconstructions for *Leukoma antiqua* (which could be considered as synonymous of *A. antiqua*, Pérez et al., 2013) will be limited until the 15 years of life because growth rates are sharply reduced. Therefore, the isotope samples in the external marginal zone of the shell can not be considered particularly informative in terms of stable isotopes.

Although it would be necessary to increase the number of samples of each deposit so as to better interpret the environment some general consideration can be made on the basis of the obtained data. In general, in the Southern Hemisphere, the timing and character of Quaternary interglacial climate is a combination of global and regional/local events. In particular, the interglacial environment in the Patagonia region seems to be associated with: (1) changes in the westerlies and in the position of the southern border of the semipermanent Atlantic and Pacific anticyclones and (2) the Intertropical Convergence Zone (ITCZ) (Compagnucci, 2011), the Brazil-Malvinas confluence zones (Laprida et al., 2011), and westerlies (atmospheric changes) coupled with the Antarctic Circumpolar Current (ACC, oceanographic changes) (Lamy et al., 2010; Fletcher & Moreno, 2011). However, patterns of climate variability on a multicentennial to millennial scale have been linked to the onset of the modern state of the ENSO (Lamy et al., 2002; Lamy & de Pol-Holz, 2013). In addition, the topographical and bathymetrical settings of the San Jorge Gulf result in complex local oceanographic conditions characterized by interacting water masses (Krock et al., 2015).

- The Present day *A. antiqua* shell. The shell length of this specimen is smaller than that of the fossil samples, and its ontogeny (10 years) is also lower in relation to the other shells. The $\delta^{18}O_{shell}$ profile has a lower $\delta^{18}O_{shell}$ (-1.47‰) value in the warmest months compared to the fossil specimens, which marks an unusual year in relation to the $\delta^{18}O_{shell}$ mean recorded (0.31‰). The maximum $\delta^{18}O$ is 1.57‰, and $\Delta\delta^{18}O$ is 3.04‰, which corresponds to a change of ca. 13°C in the seasonal temperature estimate of sea water. These results can be compared to the oxygen isotope analyses performed by Rubo et al. (2018) on *A. antiqua* modern shells from Caleta Olivia, southern San Jorge Gulf, where the mean $\delta^{18}O_{shell}$ is -0.15‰ (considering nine samples, Appendix), showing an interannual variation between -0.82‰ and 1.04‰. As previously discussed there are some offsets between the predicted SST and the SST recorded in Bahía Bustamante; $T_{\delta 18O}$ was overestimated since it presented a mean offset of 3.5° C, corresponding to an average of $\delta^{18}O_{shell}$ -0.80‰. Similarly, Rubo et al. (2018) estimated SSTs from *A. antiqua*; the results indicated that multi-year temperature curves computed from $\delta^{18}O_{shell}$ values overestimated instrumental temperatures. These authors recommended to adjust the $\delta^{18}O_{shell}$ values of -0.9 ±0.3‰ from expected oxygen isotopic equilibrium with the water of the shell formation to calculate past water temperatures. Therefore, on the basis of this correction, the predicted SST of our Present day shell shows values closer to those recorded by MODIS data in the region (Appendix). Another Patagonia species was analyzed throughout the oxygen isotopic shell profile. Bayer et al. (2013) indicated an interannual variation between -0.66‰ and 1.56‰ for *Amiantis purpurata* modern shells from the San Matías Gulf (Northern Patagonia, Argentina), and recorded a range of $\Delta\delta^{18}O_{shell}$= 2.22‰. The predicted seasonal SST was within the range of the SST measured in Bahía Bustamante.

- The Holocene (7.284 ±140 yr BP) *A. antiqua* shell. The $\delta^{18}O_{shell}$ profile clearly indicates the presence of marked seasonality. The annual mean $\delta^{18}O_{shell}$ is 0.43‰, the predicted mean SST is 15°C, and the seasonal change is around 10°C. Considering the offset adjustment proposed by Rubo et al. (2018), the estimated mean SST is 9°C, the minimum value is 4°C and the maximum value is 14°C. If we compared this data with the Present day sample, the SSTs would be lower. According to the dating of the outcrop where this sample was collected (Schellmann & Radtke, 2000), the oxygen isotope profile would correspond to the climatic data analyzed by other studies for the early Holocene in the Patagonian region (Renssen et al., 2005; Wagner et al., 2007; Compagnucci, 2011; among others). This indicates that the average annual temperatures would not have been higher between 9.000 and 6.000 yrs BP, since warmer conditions were recorded between 6.000 and 3.000 yrs BP, when the summer temperatures would have reached 3°C more than the estimated average temperatures for the preindustrial period (Renssen et al., 2005).

- The MIS 5 *A. antiqua* shell. Seasonality in this specimen, which is the largest of the specimens analyzed, is evident throughout the 14 years. The seasonal variation recorded was around $\Delta\delta^{18}O_{shell}$ = 2.95‰, resembling the one obtained for the Present day shell. The interglacial MIS 5e has been documented as one the warmest from the Late Quaternary (Shackleton et al., 2003; Sidall et al., 2007; Negri et al., 2015; Shakun et al., 2015, among others). However, the positive trend of the oxygen curve indicates colder seasonal temperature cycles compared to the data from the other $\delta^{18}O$ profiles. As there is not $\delta^{18}O_w$ data available for the San Jorge Gulf during this interglacial period, we used the $\delta^{18}O_w$ = -0.45‰ V-SMOW estimated by Shakun et al. (2015) for the global ocean. The annual average paleotemperature was around 15°C, with a minimum of 9°C and a maximum of 21°C. However, by applying the offset adjustment (Rubo et al. 2018), the annual mean SST was 11°C, while the extreme values were 5°C and 18°C. On the other hand, if we calculate the paleotemperatures considering the estimated $\delta^{18}O_w$ = 1.2‰ V-SMOW obtained by Wefer et al. (1996) for the South Atlantic Ocean during the interglacial-glacial Pleistocene cycles, the annual average paleotemperature was around 18°C, with a minimum of 12°C and a maximum of 25°C. These temperatures are particularly high if compared to the present day condition. From these calculations we can speculate that a global estimation of sea water isotopic composition is unable to provide a correct picture of the local conditions. Indeed, changes in local salinity might have occurred in the Malvinas (Falkland) current, owing to the very different general astronomical configuration of the MIS5 compared to the Holocene (Berger & Loutre, 1991). In addition, when Ponce et al. (2011) modelled the Patagonian Atlantic coast, they concluded that Bahía Bustamante would not have been formed as such, and the study area would have been linked to the open sea and therefore would have been directly affected by the ocean circulation conditions occurring in the South Atlantic. The bay would have originated after the Last Glacial Maximum (LGM) (Ponce et al., 2011). In this respect, the following tentative explanation is proposed. The Brazil (BC, warm) and Malvinas (MC, cold) confluence zone (BMCZ) currently stands at 29°-49° S (Stramma & England, 1999; Laprida et al., 2011). Laprida et al. (2011) reconstructed the possible displacement of the BMCZ during the Middle Pleistocene glacial periods MIS 8 and MIS 6. Such displacement would have moved about 10° north with respect to the current position. We can hypothesize that during the interglacial cycles the BMCZ would have moved further south than its current position on account of the important latitudinal changes occurred during the Pleistocene interglacials (Wefer et al., 1996), especially during MIS5e, thus causing strong positive anomalies in the SST in the Patagonian region. When the BC and the MC collide, the flow veers sharply southeastward into the South Atlantic, forming a large area of intense surface mixing of tropical and subantarctic waters characterized by the formation of meanders, eddies and filaments (Bianchi et al., 1993; Wilson & Rees, 2000; Laprida et al., 2011). The BC dominates the MC and forces it into a cyclonic loop before both currents flow to the southeast, forming a broad subtropical convergence zone, the BMCZ (Gordon, 1989; Peterson & Stramma, 1991; Laprida et al., 2011). The BC would thus cause an increase in $\delta^{18}O$ of seawater caused by changes in salinity associated with evaporation processes connected, in turn, to a rise in SST (Stanley, 1989; Murray-Wallace & Belperio, 1991; Williams et al., 1994; Brown & Lomolino, 1995; Zazo, 1999; Martinez et al., 2001). This $\delta^{18}O$ behaviour is observed nowadays in warm tropical and subtropical regions (Krantz et al., 1987; Aguirre et al, 2001; Lécuyer et al., 2004; Azzoug et al., 2012). Moreover, Bayer et al. (2013) and Bayer (2014) recorded a $\delta^{18}O$ seasonal variation of around $\Delta\delta^{18}O$ = 2.92‰ in *Amiantis pupurata* shells from the San Matías Gulf.This value is similar to that obtained in *A. antiqua* shells, ranging from -0.93‰ to 0.85‰. These authors also proposed a higher SST than that prevailing today.

- The MIS 7 *A. antiqua* shell. The $\delta^{18}O_{shell}$ profile of the specimen shows defined seasonal cycles, as in the other samples, with a mean $\delta^{18}O_{shell}$ value of 0.13‰. Its ontogeny is 15 years, similar to that of the other fossil samples. The oxygen isotope data would suggest a further drop in temperature during the cold seasons, taking into consideration the annual average value. Marked negative peaks that probably indicate markedly warmer conditions during the warm season follow the pattern of the other samples. The paleotemperature data calculated on the global $\delta^{18}O_w$ estimated by Shakun et al. (2015) indicated a predicted mean SST of 17°C, with a minimum of around 11°C and a maximum of around 22°C. When considering the offset correction (Rubo et al. 2018), the estimated SSTs are: mean SST of 13°C, a minimum value of 7°C and a maximum value of 18°C. Very warm values are obtained if we consider the $\delta^{18}O_w$ (1.2‰) estimated by Wefer et al. (1996). The MIS 7 mean $\delta^{18}O_{shell}$ value is the lowest observed with respect to the other samples, and even the peaks indicating the warm season are the most negative. The seasonal variation ($\Delta\delta^{18}O$ = 2.52‰) is not as great as in the MIS 5 and Present day specimens. There is little knowledge of the development of this interglacial cycle in Patagonia, and for this reason, the preliminary data obtained for the MIS 7 sample are difficult to compare. On the basis of geometric morphometric studies Boretto et al. (2014) reported that *A. antiqua* shells belonging to the MIS 7 interglacial have shown analogies in their configuration (size and shape) with shells from the MIS 5 deposits of Bahía Bustamante, both of which are very different in shape and size from the Holocene and modern shells.

7. FINAL REMARKS

These preliminary oxygen isotope analyses on *A. antiqua* specimens conceived as exploratory study may help determine whether $\delta^{18}O_{shell}$ behaviour and ontogeny have a direct relationship with the environmental variables of SST, SSS and $\delta^{18}O_w$ of the study area. However, to calculate the SST from the $\delta^{18}O_{shell}$, it would be necessary to monitor more live specimens and to update the environmental variables (especially the annual or seasonal oxygen isotope composition of the seawater). This would help to understand the isotopic fractionation of this species along the Patagonian coast.

The age of *A. antiqua* can be determined by counting the annual growth rings on the external shell. These conspicuous dark lines are associated with the cold season (in which the organism decreases the energy spent on growth). Seasonal temperature variability was determined over the four different geological time intervals: Present day, Holocene (7.284 ±140 yrs BP), MIS 5 and MIS 7. The isotopic profiles indicated that the oxygen isotope ratio is a powerful tool for estimating the seasonal cycle during the life of *A. antiqua.*

Assuming a minor change in salinity during the different seasons the maximum seasonal variability is ca. 13°C for the Present day and MIS 5 specimens, and ca. 9°C and 11°C for the Holocene and MIS 7 respectively. If we assume a similar isotopic composition of sea water for the Holocene sample, the average temperature will be similar to the one obtained for the modern sample (although in this case the warm season temperatures would have been lower). Temperature estimations based on the suggested water isotopic composition of the ocean during the MIS 5 and MIS 7 seem to yield unrealistically high temperatures (Wefer et al., 1996), while more recent estimations (Shakun et al., 2015) gave significantly lower temperatures. General changes in the position of the warmer and saltier Brazilian superficial current and the fresher and cooler Malvinas current cannot however be ruled out for these Pleistocene interglacials,. The estimated paleotemperatures for the MIS 5 and MIS 7 are difficult to confirm with precision because of the unknown $\delta^{18}O_w$ for the Patagonian region. It would be interesting to continue this line of research and to consider *A. antiqua* fossil shells as potential proxies for paleoenvironmental and paleoclimate reconstruction of the Patagonian coast during the Quaternary.

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