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1 **Shell sclerochronology and stable isotopes of the bivalve *Anomalocardia flexuosa* (Linnaeus, 1767)**
2 **from southern Brazil: implications for environmental and archaeological studies**

3
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25
26 **Abstract**

27 We conduct the first stable isotopic and sclerochronological calibration of the bivalve *Anomalocardia*
28 *flexuosa* (Linnaeus, 1767) in relation to environmental variables in a subtropical coastal area of southern
29 Brazil. We investigate incremental shell growth patterns and $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of modern specimens
30 collected alive from the Laguna Lagoonal System (LLS). As shells of *Anomalocardia flexuosa* are also the
31 main components of pre-Columbian archaeological shell mounds and middens distributed along the
32 Brazilian coastline, late Holocene archaeological specimens from a local shell mound (Cabeçuda) were
33 selected to compare their stable carbon and oxygen isotopes with those of modern specimens. Shell
34 growth increments, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values respond to a complex of environmental conditions, involving,

35 for example, the effects of temperature and salinity. The isotopic information extracted from
36 archaeological specimens from Cabeçuda shell midden in the LLS indirectly indicates that environmental
37 conditions during the late Holocene were different from present day. In particular, intra-shell $\delta^{18}\text{O}$ and
38 $\delta^{13}\text{C}$ values of archaeological shells reveal a stronger marine influence at 3 ka cal BP, which is in contrast
39 to the seasonal freshwater/seawater balance that currently prevails at the LLS.

40

41 **Key words:** Eastern South America; Coastal lagoon; *Anomalocardia flexuosa* shells; Sclerochronology;
42 Light stable isotopes

43

44 **Introduction**

45 Highly-resolved palaeoenvironmental information for tropical and subtropical coastal areas of South
46 America predominantly come from pollen records, calcareous nanofossil assemblages and
47 geomorphological evidence (e.g. Baker and Fritz, 2015; França et al., 2013; Gyllencreutz et al., 2010).
48 Whereas these records provide robust palaeoclimate and palaeoenvironmental information spanning
49 decadal to millennial timescales, there is still a need for archives resolving sub-annual environmental
50 conditions (e.g. Carré et al., 2005; Yan et al., 2012). For example, data on intra-annual sea surface
51 temperature and biological productivity are crucial for assessing the impact of extreme ocean-
52 atmosphere phenomena, such as the El Niño/Southern Oscillation, on local/regional hydrological and
53 biological processes at seasonal time-scales (Aravena et al., 2014; Garcia et al., 2003; Stenseth et al.,
54 2002). Furthermore, several lines of evidence point to considerable reorganisations of coastal
55 ecosystems from the middle Holocene to present-day in response to relative sea-level changes in
56 eastern South America (Angulo et al., 2006). In some tropical and subtropical areas geomorphological
57 and palynological records reveal a marked retraction or disappearance of rich aquatic ecotones, such as
58 estuaries and coastal lagoons, during this period (Carvalho do Amaral et al., 2012; Carvalho et al., 2004;
59 Fornari et al., 2012; França et al., 2013). Although it is well known that present day human populations
60 inhabiting these areas are extremely vulnerable to increasing climate and environmental variability
61 (Defeo et al., 2013; Magrin et al., 2007), the impact of these changes on human societies in the past
62 remains largely unknown.

63 Aquatic mollusc shells are excellent archives for high resolution palaeoclimate reconstructions. During
64 carbonate precipitation, mollusc shells register endogenous and environmental information in the form
65 of geochemical signatures and structural characteristics spanning daily to annual time intervals (e.g.
66 Goodwin et al., 2003; Schöne, 2008; Schöne and Surge, 2012). Mollusc shells can provide information on
67 water temperature, hydrologic balance, productivity and ocean circulation (Dettman et al., 2004; García-
68 March et al., 2011; Mannino et al., 2008; Milano et al., 2016; Prendergast et al., 2013; Schöne and

69 Gillikin, 2013; Surge et al., 2003; Wanamaker et al., 2008). Moreover, many intertidal and subtidal
70 species have been widely exploited by human populations in the past and thus their shells are often
71 preserved in archaeological sites (Colonese et al., 2011; Erlandson, 2001; Gaspar et al., 2008), offering
72 the opportunity to investigate past climate and environmental conditions in relation to human societies.
73 Finally, many species exploited in the past are still economically relevant to present-day human societies
74 (Bardach, 1997), as such an increasing knowledge of their physiology and ecology is vital for
75 implementing appropriate management and assessment strategies.

76 In the present study we conduct the first stable isotopic and sclerochronological calibration of the
77 bivalve *A. flexuosa* (Linnaeus, 1767) in relation to environmental variables. We investigate incremental
78 shell growth patterns and stable carbon and oxygen isotope composition of modern specimens collected
79 alive from the Laguna Lagoonal System (LLS), in southern Brazil. *A. flexuosa* has considerable economic
80 importance for present-day coastal communities along the subtropical and tropical Atlantic coasts of
81 South America (Boehs et al., 2008; Gaspar et al., 2011; Silva-Cavalcanti and Costa, 2011). Its shells are
82 also very abundant in pre-Columbian shell mounds locally known as *sambaquis* found in these regions
83 (Gaspar et al., 2008). Thus, we also selected archaeological specimens from a local shell mound to
84 compare their oxygen and carbon isotopic profiles with modern counterparts. Our main aim is to
85 establish a new environmental proxy for subtropical coastal lagoon and estuaries in eastern South
86 America, with a secondary aim of exploring seasonal environmental conditions in pre-Columbian times,
87 during the expansion of sambaqui cultures.

88

89 **Environmental and archaeological setting**

90

91 *Laguna Lagoonal System*

92

93 The Laguna Lagoonal System (LLS) is located in the State of Santa Catarina, in the southern coast of
94 Brazil (S 28°12' E 48°38'; Fig. 1). The study area has a temperate climate (Peel et al., 2007) with mean air
95 temperatures around 13 °C in the winter (July) and 22 °C in the summer (January) (De Pellegrin et al.,
96 2014), and mean annual rainfall between 1250 mm, in the north, and 1400mm, in the south (Orselli,
97 1986). The LLS is a choked lagoon complex (Kjerfve, 1994) composed of three lagoons covering an area
98 of 184 km²: Mirim, in the north; Imaruí, the largest lagoon in the middle of the system; and Santo
99 Antônio, in the south (Fig. 1). The LLS is connected with the adjacent ocean by a narrow inlet in the
100 south eastern sector of the system (Entrada da Barra), at Santo Antônio Lagoon. Freshwater inputs
101 derive from three main rivers (Tubarão, Duna and Aratingaúba), however the largest contribution is
102 provided by Tubarão River with a drainage area of 4.728 km² and an average annual discharge of 50 m³/s

103 (Fonseca and Netto, 2006), forming a lagoonal delta into Santo Antônio Lagoon (Giannini et al., 2010;
104 Fornari et al., 2012). The Tubarão lagoonal delta has gradually silted the lagoon complex to the south of
105 Santo Antônio Lagoon since the mid Holocene (Giannini et al. 2007, 2010; Fornari et al., 2012). A sand
106 barrier delimits the LLS to the east, whereas the western side is bordered by the pre-Cenozoic crystalline
107 rock basement, a geomorphological unit known as the Serra do Tabuleiro (Hesp et al., 2009). The mean
108 depth of the lagoon is around 2 m, and circulation is driven by a complex interaction of wind, tide and
109 freshwater discharge (Fonseca and Netto, 2006).

110

111 *Early fisheries of Atlantic South America: the sambaquis*

112

113 The coastal areas of eastern South America have supported human occupation at least since the middle
114 Holocene (Lima, 2002) and from ca. 6 to 1.5 ka cal BP pre-ceramic coastal populations occupied the
115 surrounding areas of the LLS. These groups were highly adapted to interact with a variety of coastal
116 environments and left behind some of the world's largest shell mounds, or sambaquis (DeBlasi et al.,
117 2007; Gaspar et al., 2008; Giannini et al., 2010). These sites are frequently stratified and predominantly
118 composed of shells, notably of *A. flexuosa*, and fish bones (Villagran, 2014). Recent studies have attested
119 that some sites were occupied for hundreds of years (Gaspar et al., 2008), with shells intentionally used
120 as raw material for mound construction, which in some cases reached more than 30m high and several
121 hundred meters in diameter (DeBlasis et al., 1998; 2007). The sambaquis had distinct functions, from
122 burial sites (with hundreds of human burials) to dwelling structures (Villagran, 2014), and indirectly
123 testify to a large-scale, long-term exploitation of coastal resources. Multidisciplinary studies confirm that
124 the builders of the sambaquis were involved in fishing, especially in bays and coastal lagoons (Figuti,
125 1993; Villagran et al., 2011; Colonese et al., 2014; Bastos et al., 2015), and complemented their diet with
126 plants and terrestrial mammal resources (Scheel-Ybert, 2001). Other elements of the material culture
127 similarly invoke the reliance on marine resources, such as tools made of sea mammal and fish bones, as
128 well as sophisticated zoomorphic sculptures representing aquatic animals (Gaspar et al., 2008). The
129 emergence and maintenance of this long-term cultural practice required a deep understanding of
130 coastal environmental conditions and their changes through time.

131

132 *Anomalocardia flexuosa, Mollusca, Bivalvia, Veneridae*

133

134 The bivalve *A. flexuosa* (Linnaeus, 1767), formerly known as *A. brasiliiana* (Gmelin, 1791), inhabits
135 shallow subtidal and intertidal areas of transitional environments (e.g. estuaries, coastal lagoons) from
136 the Caribbean to subtropical South America (e.g. Monti et al., 1991; Rios, 1994; Rodrigues et al., 2013;
137 Silva-Cavalcanti and Costa, 2011). It occurs predominantly in fine sand or a mixture of sand and mud

138 substrates, and in the study area the species was observed only in the sandy sediments of the eastern
139 portion of the Santo Antônio Lagoon. The species typically tolerates large variations in salinity (Leonel et
140 al., 1983; Monti et al., 1991; Rodrigues et al., 2013) and has a short lifespan of ca. 2 – 3 years (Monti et
141 al., 1991; Rodrigues et al., 2013). In southern Brazil, this species attains an average adult size of ca. 30
142 mm, but larger individuals have previously been reported (e.g. Boehs et al., 2008). In latitudes marked
143 by minimal temperature variation the reproductive cycle is continuous throughout the year (e.g. Boehs
144 et al., 2008; Luz and Boehs, 2011), but peaks have been recorded in the spring, summer and autumn,
145 with well-defined growth cessation in the winter at mid-latitudes (Barreira and Araujo, 2005; Luz and
146 Boehs, 2011).

147

148 **Material and methods**

149

150 *Monitoring environmental parameters*

151

152 Surface temperature (ST, °C), surface salinity (SS, PSU) and $\delta^{18}\text{O}$ of the water ($\delta^{18}\text{O}_w$, V-SMOW) were
153 measured at seasonal and monthly intervals for one year, from August 2008 to August 2009, in several
154 areas of the LLS and in the adjacent open sea (Fig. 1). ST and SS were measured at approximately 10 cm
155 below the water surface using an YSI 556 multiparameter probe. Seasonal and monthly samples of
156 surface water (10 ml) were collected for oxygen isotopic analysis. Seasonal water samples (austral
157 winter, spring, summer and autumn) were taken to establish the $\delta^{18}\text{O}_w$ values of the main sources of
158 freshwater (Tubarão, Duna and Aratingaúba) and seawater input within the LLS, and their relation with
159 SS. Monthly water samples were collected to examine the $\delta^{18}\text{O}_w$ values in areas where living *A. flexuosa*
160 were collected for shell isotope analysis. Daily ST and SS were also recorded every 36 min at the Marine
161 Science Laboratory (Universidade do Sul de Santa Catarina) at the Santo Antônio Lagoon (Fig. 1, n. 15),
162 using the same instrumentation as reported above. An Inverse-Distance-Weighting-Method (IDW) was
163 used in ArcGIS to explore spatial variability in SS and $\delta^{18}\text{O}_w$ based on data collected at the seasonal
164 scale. Monthly data on Chlorophyll *a* was obtained from Meurer and Netto (2007), who measured the
165 primary productivity in several locations of the Santo Antônio Lagoon in 2007. Precipitation values for
166 the study area (year 2008-2009) were kindly provided by Epagri/CIRAM
167 (<http://www.inmet.gov.br/portal/>). Astronomic tidal oscillations for Laguna between August 2008 and
168 2009 were simulated using the free software WXTide32 (<http://www.wxtide32.com/>). The tidal
169 simulation does not include weather effects.

170

171 *Shell preparation for sclerochronological and stable isotope analyses*

172

173 From a depth of ca. 50 cm, twelve living specimens of *A. flexuosa* were collected on the 15th of July,
174 2009, from areas 11, 12, 13, 14, and 16 (Fig. 1) of Santo Antônio Lagoon. Immediately after collection
175 the soft parts were removed to prevent the animals from secreting additional shell carbonate. Shell
176 preparation for incremental analysis was performed at the INCREMENTS Research Group of the
177 University of Mainz (Germany). After rinsing and air-drying, the shells were partially embedded in an
178 epoxy resin, then sectioned perpendicularly to the growth lines (from the umbo to the ventral margin;
179 Fig. 2A-C) with a 0.4 mm thick diamond-coated saw blade mounted to a low speed saw (Buehler, IsoMet
180 1000). From each shell, two slabs of 3 to 5 mm thickness were then cut and glued to a glass slide, ground
181 on glass plates with F800 and F1200 grit SiC powder and polished with 1 μm Al_2O_3 powder. For each
182 shell, one thick-section was used for growth increment analysis, whereas the other was selected for
183 stable isotope analyses. Samples for the analysis of growth increments were immersed in Mutvei's
184 solution (see Schöne et al., 2005 for details), which gently etches the calcium carbonate while preserving
185 the organic matrix and dyeing the sugars and glycoproteins with Alcian blue. The shell structure of *A.*
186 *flexuosa* is formed by an outer crossed-lamellar layer (CL) that becomes homogeneous inwards (Taylor
187 et al., 1973). The use of Mutvei's solution in the CL emphasizes the organic-rich growth lines which
188 appear dark blue, whereas the carbonate-rich growth increments appear light blue. This allows shell
189 increments to be easily distinguished by microscopic analysis. Shell slabs were analysed with a Keyence
190 VHX-100 digital microscope at different magnifications (from 300 to 500) in the Department of
191 Archaeology at the University of York. Incremental width was measured in the direction of growth in the
192 outer shell layer (Fig. 2C-E).

193 Three archaeological shells (CAB1, CAB2, CAB3) were sampled from an archaeological shell mound,
194 Cabeçuda (Fig. 1, n. 12), to compare with data obtained from the modern specimens. The archaeological
195 shells from Cabeçuda were associated with a human burial (Burial 15) dated between 3,235 and 3,070
196 calibrated years before present (2σ , AMS, Beta - 383566; Farias and DeBlasis, 2014). The archaeological
197 shells were prepared for stable isotope analysis following the same procedure as the modern
198 specimens.

199 The shell mineral composition was investigated using X-ray diffraction (XRD), with a mixture of calcite
200 and aragonite standards. Raman spectroscopy was used to increase the accuracy of mineralogical
201 identification of the outer crossed-lamellar layer, as well as to investigate the nature of the pigments
202 that were visible on the section of some specimens. Raman spectra were obtained using an HORIBA
203 XploRA Raman microscope using a x50 long working distance objective (NA = 0.50) in confocal mode
204 with 532 nm laser wavelength. The shell was sectioned and polished to enable the collection of Raman
205 spectra from the internal shell structure. Eleven positions on the sectioned shell were analysed with a

206 single spectrum collected in both the pigmented and non-pigmented regions (positions A to K in Fig. 3).
207 Spectra were acquired using LabSpec 5 software set at 3.8mW laser power at the sample and 1s
208 exposure with each spectrum per region averaged over 40 spectral repetitions. The software package
209 IGOR Pro. 6.32 was used to analyse the Raman spectra using Gaussian peak-fitting procedures.

210

211 *Isolating environmentally-controlled growth patterns in modern shells*

212

213 Typically, shell growth rate decreases as the bivalve grows older. This trend is superimposed by the
214 effect of environmental conditions on the animal's physiology (Schöne, 2008). In order to examine the
215 response of shell growth to environmental change, this age-related trend was removed following the
216 procedure reported in detail in Schöne (2003). In short, after measuring the single increments, the
217 exponential growth function for each shell was estimated, and then a growth index (GI) calculated by
218 dividing the measured value by the estimated growth at each measurement (ratio-based GI). A series of
219 filters (high, low and band pass) were then used to explore low, medium and high frequency signals on
220 growth incremental series (Miyaji et al., 2007; Schöne, 2013). Filters were used with a transition width
221 of 0.02, following Parks-McClellan algorithms in PAST 3.x (Hammer et al., 2001). Spectral analysis of
222 filtered increment time-series was accomplished by means of Continuous Wavelet Transformation
223 (Morlet wavelet, wavenumber 6 <http://paos.colorado.edu/research/wavelets/>) (Torrence and Compo,
224 1998; Wanamaker et al., 2008). The filtered time-series of each shell was normalized to the global
225 wavelet spectrum, and a 95 % confidence interval was applied against a red-noise (autoregressive lag-1)
226 background spectrum.

227

228 *Stable isotope analysis*

229

230 Stable isotope analyses were performed on modern specimens from area 13 (AF-13-1, AF-13-2, AF-13-3)
231 and 12 (AF-12-3), and three archaeological shells (CAB1, CAB2, CAB3). Carbonate samples (ca. 50 to 150
232 µg) were manually drilled sequentially along the umbo – ventral margin axis from the outer shell layer
233 (Fig. 2B). Samples were taken using a manual microdrill with a 0.4 mm diameter bit. Distances between
234 individual sample spots ranged from 0 to 1.1 mm.

235 Modern shell oxygen and carbon isotopic composition was measured at the Stable Isotope Facility at the
236 University of Wyoming (USA) using a Thermo Gasbench coupled to a Thermo Delta Plus XL IRMS, after
237 reaction with 99.99% H₃PO₄ (100µL) at 25 °C for 24 hours. Isotope data were normalized against
238 calibrated NBS19 in-house standards, UWSIF18 ($\delta^{18}\text{O} = -3.3 \text{ ‰}$, $\delta^{13}\text{C} = +2.6 \text{ ‰}$) and UWSIF06 ($\delta^{18}\text{O} = -$
239 28.9 ‰ , $\delta^{13}\text{C} = +11.6 \text{ ‰}$), with 1 σ external reproducibility and average internal precision of 0.2 ‰ and
240 0.15 ‰ for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ respectively. Archaeological samples were analysed at the University of Mainz

241 (Germany) on a Thermo Finnigan MAT 253 continuous flow IRMS, coupled to a Gas Bench II, after
242 reaction with 99.99% H₃PO₄ at 72 °C for 2 hours. Isotope data were calibrated against a NBS 19
243 calibrated Carrara marble standard ($\delta^{18}\text{O} = -1.9 \text{ ‰}$, $\delta^{13}\text{C} = +2.0 \text{ ‰}$), with 1 σ external reproducibility and
244 internal precision better than 0.06‰ and 0.04‰ for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ respectively.

245 Oxygen isotopic composition of sampled water was analysed via equilibration with CO₂ at the
246 Geochronological Research Center (CPGeo) of the Universidade de São Paulo (Brazil) using a DeltaPlus
247 Advantage (Thermo Finnigan) IRMS. Analytical precision for was better than 0.07 ‰ for water $\delta^{18}\text{O}$.

248 Results are reported in δ -notation, and $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values are given as parts per mil (‰). Shell $\delta^{18}\text{O}$
249 and $\delta^{13}\text{C}$ values are reported to V-PDB, whilst water $\delta^{18}\text{O}$ are reported to V-SMOW.

250 Shell $\delta^{18}\text{O}$ values reflect the temperature and $\delta^{18}\text{O}_w$ values experienced by the animal during shell
251 growth, which is in turn regulated by the animal's physiological tolerance to environmental conditions
252 and endogenous controls (Schöne, 2008). In order to assess the range of environmental conditions
253 experienced by *A. flexuosa* at the LLS we compared the shell $\delta^{18}\text{O}$ values with highly resolved predicted
254 aragonite $\delta^{18}\text{O}$ values in isotopic equilibrium with instrumentally measured ST and $\delta^{18}\text{O}_w$ values for the
255 study area over one year. We used our locally established $\delta^{18}\text{O}_w$ -SS relationship to derive the $\delta^{18}\text{O}_w$
256 values from sub-daily SS data. The SS-derived $\delta^{18}\text{O}_w$ values and the measured STs were then used to
257 calculate the shell $\delta^{18}\text{O}$ values according to the empirically derived temperature equation obtained by
258 Grossman and Ku (1986). The equation was slightly modified to convert the $\delta^{18}\text{O}_w$ from V-SMOW to V-
259 PDB (Dettman et al., 1999):

260

$$261 \quad T \text{ (}^\circ\text{C)} = 20.6 - 4.34(\text{shell } \delta^{18}\text{O} - (\delta^{18}\text{O}_w - 0.27)) \quad (\text{Eq. 1})$$

262

263 According to this equation, a 1 ‰ change in shell $\delta^{18}\text{O}$ values corresponds to a change in water
264 temperature of 4.34 °C, providing that the $\delta^{18}\text{O}_w$ remains unchanged. This was not the case for the
265 study area (see below). Coastal areas affected by freshwater input have variable $\delta^{18}\text{O}_w$ values that may
266 complicate palaeotemperature estimations if precise temperature and $\delta^{18}\text{O}_w$ values are not known (e.g.
267 Ingram et al., 1996; Dettman et al., 2004).

268 The main source of carbon isotopes in bivalve shells is dissolved inorganic carbon (DIC) (e.g. Gillikin et
269 al., 2006; McConnaughey and Gillikin, 2008; Poulain et al., 2010). Compared to seawater, DIC is typically
270 ¹³C-depleted in lacustrine and estuarine environments due to the larger amount of CO₂ derived from
271 decaying continental organic matter, which often has distinct $\delta^{13}\text{C}$ values (e.g. C3 and C4 plants; O'Leary,
272 1988), and the dissolution of carbonates (Mook and Tan, 1991). Consequently, freshwater and estuarine
273 molluscs tend to have comparatively lower shell $\delta^{13}\text{C}$ values than their marine counterparts (Dettman et
274 al., 1999; Gillikin et al., 2009). Since the $\delta^{13}\text{C}$ values for the local DIC were not available, we estimated

275 the stable carbon isotope composition for the DIC ($\delta^{13}\text{C}_{\text{DIC}}$) using data collected from Barros et al. (2010)
276 in Babitonga Bay, approximately 200 km north of the study area. The $\delta^{13}\text{C}_{\text{DIC}}$ values from Babitonga Bay
277 are positively correlated with local SS ($R^2 = 0.7$), and represented by the following $\delta^{13}\text{C}_{\text{DIC}}$ -SS relationship:

$$\delta^{13}\text{C}_{\text{DIC}} (\text{‰}) = 0.2 * \text{SS (PSU)} - 8.1 \quad (\text{Eq. 2})$$

281 Results

282 *Environmental conditions at Santo Antônio Lagoon*

283
284 The daily ST from Santo Antônio Lagoon (area 15; Fig. 1) in the southern sector of the LLS show clear
285 seasonal variation (15°C), ranging from 29°C in summer (January-February) to 14°C in winter (July-
286 August) (Fig. 4A). Similarly, the daily SS exhibits a strong seasonal oscillation, ranging from 0 to 35 PSU in
287 spring-summer (October-March) and autumn-winter (May-August), respectively (Fig. 4B). Seasonal
288 changes in SS respond to variations in precipitation over the study area, which is higher in spring-
289 summer compared to autumn-winter (Fig. 4C). High frequency variation in SS and ST values (daily,
290 weekly) instead reflects the effect of mixed semidiurnal tide on the freshwater/seawater circulation (Fig.
291 4D). The highest tides facilitate the input of seawater within the LLS, while the opposite occurs with the
292 lowest tides.

293 The $\delta^{18}\text{O}_w$ values of samples collected at seasonal and monthly intervals ($n = 109$) ranged from $+0.1 \text{‰}$
294 to -6.4‰ . As expected, a clear isotopic gradient was observed from seawater to freshwater
295 endmembers, with average values ranging from $-0.9 \pm 0.9 \text{‰}$ in seawater ($n = 4$), to $-3.7 \pm 0.7 \text{‰}$ in the
296 Duna River ($n = 4$), $-4.4 \pm 0.9 \text{‰}$ in the Aragatingaúba River ($n = 4$), and $-5.0 \pm 0.9 \text{‰}$ in the Tubarão River
297 ($n = 4$). Whilst a significant statistical difference was observed for the average $\delta^{18}\text{O}_w$ values between
298 seawater and freshwater ($p < 0.001$, $F = 12.36$, One-way ANOVA), no differences were observed
299 between the rivers ($p < 0.578$, $F = 0.58$). However, the $\delta^{18}\text{O}_w$ values also changed at the seasonal scale
300 (by 6.5‰). Higher and lower $\delta^{18}\text{O}_w$ values were recorded in winter-early spring (August-September) and
301 summer-early autumn (February-April), respectively, tracking generally the salinity distribution (Fig. 5A-
302 B). The $\delta^{18}\text{O}_w$ values showed a moderately positive correlation with the SS values within the LLS ($R^2 =$
303 0.5 ; $p < 0.001$; Fig. 6):

$$\delta^{18}\text{O}_w (\text{‰}) = 0.10 * \text{SS (PSU)} - 4.0 \quad (\text{Eq. 3})$$

$$R^2 = 0.5; p < 0.001$$

307

308 As a result of the seasonal variation in ST and $\delta^{18}\text{O}_w$, the predicted shell $\delta^{18}\text{O}$ values (Fig. 4E) showed a
309 quasi-sinusoidal trend, ranging from +0.4 ‰ to -6.3 ‰ in winter and spring-summer respectively, with
310 an annual average value of -2.3 ± 1.4 ‰. A similar seasonal trend was observed for the estimated
311 $\delta^{13}\text{C}_{\text{DIC}}$, with lower (-8.1 ‰) and higher (-1.1 ‰) values in spring-summer and autumn-winter
312 respectively (Fig. 4F). Chlorophyll *a* showed a seasonal trend with higher values in summer (ca. 20,000
313 mg/m^3) and lower concentrations in autumn and winter (ca. 4,000 mg/m^3) (Fig. 4G).

314

315 *Mineral and organic composition of the outer shell layer*

316 Both Raman spectroscopy and X-ray diffraction (not shown) indicate that shell of *A. flexuosa* is made of
317 aragonite. The aragonite signature was identified in the Raman spectra by comparing the peak positions
318 at 150, 204, 700 and 1081 cm^{-1} (the latter being the main carbonate ν_1 band) to other literature (e.g.
319 Urmos et al., 1991). Raman spectroscopy also revealed that pigmented regions in the outer shell layer of
320 the sampled specimen were carotenoids (Fig. 7). The two prominent peaks in the carotenoid signature,
321 ν_3 and ν_1 , are related to the in-phase stretching of the double and single carbon-carbon bonds in the
322 main polyene chain, respectively (Withnall et al., 2003). The peak intensity of the ν_3 and ν_1 bands
323 increases, with the ν_1 peak position shifting to lower wave numbers as a function of the increasing (i.e.,
324 deepening) pigmentation (Fig. 7).

325 The peak position of the ν_1 Raman band is related to the number of conjugated double bonds in the
326 main polyene chain of the carotenoid and can be used to identify the type of carotenoid in the shell
327 (Withnall et al., 2003). In the case of the *A. flexuosa* shell, two types of carotenoids were identified; β -
328 carotene and decapreno- β -carotene. Carotenoids are commonly found in marine organisms (Urmos et
329 al., 1991; Withnall et al., 2003; Maoka, 2011). Since they are not synthesized *de novo* by animals their
330 presence in the shells of *A. flexuosa* can be either associated with their accumulation through the food
331 (microalgae) or through metabolic reactions (Maoka, 2011).

332

333 *Shell growth increments of modern Anomalocardia flexuosa*

334

335 The number of microgrowth lines observed in the outer layer of twelve cross-sectioned specimens of *A.*
336 *flexuosa* from Santo Antônio Lagoon ranged from 393 to 690, and showed cyclical variations (Fig. 8A-B).
337 The lines were oriented parallel to the direction of shell growth, and separated highly variable
338 microgrowth increments, with widths ranging from 1.3 to 590 μm (average 60.5 ± 46.8 μm). The
339 broadest increments were represented in the earliest portion of the shells (e.g. up to 590 μm in AF-13-1;
340 Fig. 8A) and decreased with the ontogenetic age toward the shell ventral margin (from 6.3 to 239 μm).

341 The age-detrended profile of shell growth increments allowed for a better appreciation of the variation
342 in growth rate as a function of environmental conditions (Fig. 8B). An overall decrease in the growth rate
343 was observed toward the ventral margin of specimen AF-13-1, which represents the last period of shell
344 development prior to live collection in winter 2009. The same pattern was recorded in all the specimens.
345 Reduced growth rate thus seems to correspond with low temperatures as well as low primary
346 productivity. By contrast, an overall increase in shell growth rate was observed in almost all the
347 specimens (91 %) before the decreasing trend in winter. The maximum growth rate corresponded with
348 high temperatures and occurred at the time of maximum primary productivity in spring-summer.
349 The age-detrended growth increments showed distinct periodic cycles. High and low pass filters
350 revealed significant periodicity of ca. 200 microincrements, which likely corresponds to the annual
351 growth period of 200 days (Fig. 9A-B). The low pass filter also revealed bundles with ca. 16 to 32
352 increments that are significantly detected (red noise at 95% confidence level) with a band pass filter (Fig.
353 9C). Bundles with ca. 32 increments could correspond with higher astronomic neap tides observed three
354 times a year with a frequency of ca. 32 days. Bundles with ca. 16 – 14 increments were observed in all
355 the specimens and likely correspond to spring tide-to-spring-tide-cycle (apogee) and/or full-moon-to-
356 new-moon-cycle (perigee) (Schöne and Surge, 2012). The tidal growth pattern was further corroborated
357 by the alignment between the last 200 daily increments and the daily average tidal variation of 200 days
358 prior to shell collection in winter 2009 (Fig. 9D). Tidal growth patterns could be also distinguished by the
359 periodic occurrence of broad, closely spaced daily growth lines during neap and spring tides, and were
360 particularly visible in the juvenile portion of the shell (see also Fig. 2C-E). Similar results have been
361 reported for other species from tide-controlled settings (Kanazawa and Sato, 2008; Lutz and Rhoads,
362 1977; Milano et al., 2016; Schöne and Surge, 2012).

363

364 *Shell stable isotope composition of modern Anomalocardia flexuosa*

365

366 Modern shells from Santo Antônio Lagoon had average $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values ranging from $-1.6 \pm 0.4 \text{ ‰}$
367 (AF-12-3) to $-2.2 \pm 0.7 \text{ ‰}$ (AF-13-3) and $-0.9 \pm 0.6 \text{ ‰}$ (AF-12-3) to $0.0 \pm 0.7 \text{ ‰}$ (AF-13-1), respectively.
368 Intra-shell $\delta^{18}\text{O}$ variability was highly variable among specimens (from 1.2 ‰ to 3.3 ‰ in AF-12-3 and
369 AF-13-1, respectively) and showed cyclical variations representing up to three cycles (e.g. AF-13-2). A
370 similar pattern was roughly displayed by intra-shell $\delta^{13}\text{C}$ values (from 1.9 ‰ to 2.9 ‰ in AF-13-2 and AF-
371 13-3, respectively) (Fig. 10A-D).

372 In conjunction with the sampling resolution and shell incremental record, the isotope cycles enabled us
373 to estimate the life span of analysed specimens and the shell growth rate through ontogeny. The life
374 span of the modern specimens did not exceed 2 – 3 years, with an average growth rate of 14.2 ± 4.9

375 mm/year. *A. flexuosa* grows faster in its first year of shell formation (19.2 ± 3.0 mm/year), decreasing in
376 subsequent years (12.4 ± 0.7 mm/year), with a minimum rate achieved in the last year of life (8.2 ± 3.2
377 mm/year).

378 Based on the predicted shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}_{\text{DIC}}$ values, low and high $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values in modern
379 specimens corresponded with warmer/wet (spring-summer) and colder/dry (winter) conditions. This
380 was further corroborated by moderately positive correlations between shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values for
381 most of the specimens ($R^2 = 0.3$, $p < 0.05$), except for specimen AF-12-3 ($R^2 = 0.01$, $p = 0.696$). However
382 the maximum measured intra-shell $\delta^{18}\text{O}$ range (i.e. 3.3 ‰, AF-13-1) was considerably lower than the
383 predicted annual range of $\delta^{18}\text{O}$ values (6.7 ‰). The lowest measured $\delta^{18}\text{O}$ value (-4.2 ‰, AF-13-3) was
384 higher by ca. 2.0 ‰ compared to the lowest predicted counterpart (-6.3 ‰). An offset of ca. 0.2 ‰ was
385 also observed between the highest measured ($+0.2$ ‰, AF-13-1) and predicted ($+0.4$ ‰) $\delta^{18}\text{O}$ values in
386 winter. A temporal alignment between predicted and measured shell $\delta^{18}\text{O}$ values for the last year of
387 shell formation in four specimens illustrated the magnitude of these offsets. Measured shell $\delta^{18}\text{O}$ values
388 were higher by 1.9 ‰ compared to the minimum predicted average values for spring-summer and lower
389 by 1 ‰ compared to the maximum average predicted values for winter (Fig. 11). Computing spring-
390 summer offsets into the palaeotemperature equation, we estimated that *A. flexuosa* slowed growth, or
391 stopped it, when average salinity dropped below 15.4 PSU and temperature rose above 22.4 °C. Given
392 the tolerance *A. flexuosa* to salinity values up to 42 PSU (Leonel et al., 1983), slowed growth/ growth
393 interruption in winter would most likely be a function of thermal tolerance. The winter offset revealed
394 that *A. flexuosa* slowed the growth, or stopped it, at temperatures below 18.4 °C. As such, the
395 correlation between measured and the average predicted shell $\delta^{18}\text{O}$ values for the last year of growth of
396 all the specimens was moderately weak ($R^2 = 0.3$, $p < 0.001$).

397

398 *Archaeological shells*

399

400 Archaeological specimens had average shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values ranging from -1.4 ± 0.6 ‰ (CAB2) to -
401 1.7 ± 0.5 ‰ (CAB2 and CAB3) and $+0.8 \pm 0.5$ ‰ (CAB3) to $+0.9 \pm 0.4$ ‰ (CAB1), respectively (Fig. 12).
402 Intra-shell $\delta^{18}\text{O}$ variability was very similar among specimens (from 2 ‰ to 2.5 ‰ in CAB1 and CAB3
403 respectively) and showed up to three complete cyclical oscillations (e.g. CBA3). This pattern was roughly
404 displayed by the intra-shell $\delta^{13}\text{C}$ variability (from 1.9 ‰ to 2.2 ‰ in CAB1 and CAB3, respectively), which
405 in turn correlated moderately with $\delta^{18}\text{O}$ (from $R^2 = 0.3$ to 0.4 , $p < 0.001$), except for specimen CAB3 ($R^2 =$
406 0.01 , $p = 0.122$). Similar to the modern specimens, the shell growth rate in late Holocene specimens was
407 largely influenced by seasonal temperature/salinity conditions. This was well evidenced by the

408 correspondence between growth cessation (marked by pronounced external growth checks) and peaks
409 of higher shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values in the specimen CAB3.

410 Based on winter $\delta^{18}\text{O}$ peaks (e.g. Fig. 12, CAB3), estimated annual growth rates ranged from a minimum
411 of 11.8 ± 4.8 mm/year in the first year (only partially represented in CAB1 and CAB3) to 6.4 ± 1.7
412 mm/year and 4.4 mm/year (only in CAB3) in the second and third year respectively.

413

414 **Discussion**

415

416 Similar to other choked lagoons (Kjerfve, 1994), water circulation at the LLS is driven by the complex
417 interaction between winds, tidal oscillation and seasonal runoff events. During the summer, for
418 example, the prevailing NE winds facilitate the movement of less saline water masses towards the
419 southern margins of the lagoon, consequently reducing salinity in Santo Antônio Lagoon. In winter, by
420 contrast, S-SE winds increase the input of seawater into Santo Antônio Lagoon, thus enhancing salinity
421 in the area (Fonseca and Netto, 2006). The high frequency of precipitation in spring-summer compared
422 to winter also contributes to the seasonality of SS in Santo Antônio Lagoon. Santo Antônio Lagoon's
423 $\delta^{18}\text{O}_w$ values are thus expected to reflect the hydrological balance between freshwater and seawater
424 input. The moderate positive correlation between $\delta^{18}\text{O}_w$ and SS values ($R^2 = 0.5$), however, reveals that
425 no simple relationship exists between $\delta^{18}\text{O}_w$ and SS values within the LLS. The moderate correlation can
426 be to some extent explained with changes in seasonal atmospheric circulation, which largely affect the
427 oxygen isotopic composition of precipitations in spring-summer and winter. In late summer and early
428 autumn the region is affected by the South American Summer Monsoon (Carvalho et al., 2004; Raia and
429 Cavalcanti, 2008), which transports moisture sourced from the Amazon basin, a few thousand
430 kilometres northwest. This atmospheric circulation delivers precipitation which is considerably ^{18}O -
431 depleted (ca. -7 ‰) compared to winter rainfall (ca. -3 ‰) (Cruz et al., 2005a,b; Bernal et al., 2016).
432 During the winter and early spring the region receives a larger cold-dry air mass from the mid latitude
433 South Atlantic Ocean, and heavier precipitations are formed from moisture advected from the nearby
434 Atlantic Ocean (Cruz et al., 2005a; Vera et al., 2002). The average $\delta^{18}\text{O}$ value of the precipitations (ca. -5
435 ‰) is very close to the average $\delta^{18}\text{O}_w$ value of the rivers analysed in this study (-4.4 ± 0.6 ‰). The
436 concomitance of summer freshwater input and rainfall isotopic composition was indeed recorded in
437 February 2009 at Santo Antônio Lagoon; the surface water had the minimum SS (3.0 PSU) and $\delta^{18}\text{O}_w$
438 values (-6.4 ‰), close to those of Tubarão River (2.5 PSU, -6.0‰) (Fig. 5A-B). These results thus confirm
439 that the $\delta^{18}\text{O}_w$ values at LLS are controlled predominantly by the seasonal seawater/freshwater balance,
440 along with the effect of seasonal atmospheric circulation on precipitation $\delta^{18}\text{O}$ values. The $\delta^{18}\text{O}_w$ -SS
441 relationship for the LLS is also expected to be affected by other interplaying factors, such as evaporation

442 and the catchment areas of the rivers (e.g. Lécuyer et al., 2012; Mohan and Walther, 2014), but this is
443 probably minor compared to the mechanisms described above. The effect of the mixed semidiurnal tide
444 is evident on both ST and SS profiles, from daily to monthly timescales. Similarly the estimated $\delta^{13}\text{C}_{\text{DIC}}$
445 values strongly vary as a function of seasonal changes in seawater and freshwater input.

446

447 *Shell increments and stable isotope composition of modern Anomalocardia flexuosa*

448

449 Major variations in growth increment numbers of *A. flexuosa* confirmed that the modern specimens had
450 life spans of ca. 2 to 3 years (ca. 200 days/growing season), in agreement with previous observations
451 along the Brazilian coast (Rodrigues et al., 2013). *A. flexuosa* grows fast during its first year and
452 decreases the rate through ontogeny. As a consequence the isotopic resolution also decreases in the
453 last years of shell growth. As discussed for other bivalve species (Goodwin et al., 2003), the broadest
454 range of environmental conditions experienced by *A. flexuosa* are better expressed in the earliest years
455 of shell development.

456 Age-detrended increments, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of shells of *A. flexuosa* appear to respond to
457 interplaying environmental factors. In general, higher growth rate and lower $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values
458 correspond to warmer/wet conditions in spring-summer, whereas lower growth rate and higher $\delta^{18}\text{O}$
459 and $\delta^{13}\text{C}$ values can be associated to colder/dry conditions in winter. Visible peaks relating to
460 carotenoids along the shell of some specimens (e.g. Fig. 10) predominantly correspond with periods of
461 fast growth, and could be indicative of increased metabolic activity. The occurrence of carotenoids is not
462 apparently associated with changes in shell $\delta^{13}\text{C}$ values, and this could further confirm that variations in
463 shell $\delta^{13}\text{C}$ values are mainly driven by changes in $\delta^{13}\text{C}_{\text{DIC}}$. However carotenoids are also accumulated in
464 animal gonads and are thought to be crucial for reproduction (Maoka, 2011). Visible concentrations of
465 carotenoids might thus correspond with peaks in the reproductive cycle. However, further studies are
466 required to validate this hypothesis.

467 Despite this general pattern, no significant correlations were found between average growth increments
468 and their isotopic signatures for both $\delta^{18}\text{O}$ ($R^2 = 0.00$ to 0.08 , $p = 0.92$ to 0.07) and $\delta^{13}\text{C}$ ($R^2 = 0.11$ to
469 0.00 , $p = 0.05$ to 0.82) values. The lack of significant correlations probably arises from variations in
470 sample resolution, environmental stress and endogenous mechanisms (e.g. Goodwin et al., 2003). The
471 isotopic resolution in this study ranged from 7 ± 2.6 (AF-13-1) to 9 ± 3.6 (AF-12-3) days, decreasing in
472 winter and near the shell ventral margin (up to ca. 20 days), while increasing during favourable
473 conditions in spring-summer and in the juvenile portion of the shell (up to ca. 1 day).

474 Despite the increased growth rate of *A. flexuosa* during spring-summer compared to winter, shell $\delta^{18}\text{O}$
475 values did not record the full range of temperature and salinity conditions in spring-summer. This was

476 further confirmed by episodes of reduced growth rate between peaks of maximum growth rates in
477 spring-summer (Fig. 8A-B). Slowed growth, or growth cessation, in spring-summer most likely reflects
478 the response of *A. flexuosa* to increased freshwater circulation and decreased salinity values below its
479 physiological tolerance. According to previous studies, *A. flexuosa* tolerates waters with salinity
480 conditions ranging between 17 and 42 PSU (Leonel et al., 1983; Monti et al., 1991; Silva-Cavalcanti and
481 Costa, 2011; Rodrigues et al., 2013), while at Santo Antônio Lagoon the average values were
482 considerably lower in spring-summer (ca. 5 to 10 PSU), notably from October to January (Fig. 4B). As a
483 consequence, *A. flexuosa* reduce/stop growth for days or weeks during periods of minimum salinity (<15
484 PSU). Furthermore, seawater circulation is reduced and salinity values drop considerably during low tide,
485 particularly during spring-summer. It is thus likely that in warmer/wet months *A. flexuosa* remains active
486 predominantly during the highest tides, when salinity reaches the highest values.

487 Growth cessation may also reflect the impact of sedimentation rate (Monti et al., 1991; Rodrigues et al.,
488 2013), which increases in the study area in summer (Fonseca and Netto, 2006), and is known to affect
489 molluscs in estuarine and coastal areas (Anderson, 2008; Norkko et al., 2002; Peterson, 1985). Aside
490 from these major environmental factors, growth interruption in spring-summer might be also associated
491 with spawning intervals (Schöne et al., 2005; Kanazawa and Sato, 2008), observed in *A. flexuosa* during
492 spring, summer and autumn in southern Brazilian coast (Barreira and Araujo, 2005; Luz and Boehs,
493 2011).

494 Our study indicates that *A. flexuosa* from Santo Antônio Lagoon attain the maximum and minimum
495 growth rates in spring-summer and winter respectively, as a response to seasonal temperatures and
496 possibly also nutrient availability. Shell $\delta^{18}\text{O}$ values record both temperature and salinity variations, thus
497 is a suitable candidate for palaeoenvironmental reconstructions. However, the duration and rate of shell
498 growth is strongly affected by increases freshwater circulation and decreases in salinity. As a
499 consequence *A. flexuosa* shell $\delta^{18}\text{O}$ values may not be a suitable proxy for assessing large variations in
500 the freshwater-seawater balance in the past, as the animal will stop recording these conditions below its
501 salinity tolerance (ca. 15 PSU). The moderate positive correlations between shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values
502 indicate that shell stable carbon isotope composition is driven mainly by seasonal variations in $\delta^{13}\text{C}_{\text{DIC}}$,
503 and thus is a promising proxy for salinity variations.

504

505 *Archaeological shells*

506

507 The isotopic profiles from the archaeological shells associated with the human burial dated to 3 ka cal
508 BP show some interesting differences compared to the modern counterparts. Whilst the average shell
509 $\delta^{18}\text{O}$ values were fairly comparable, archaeological specimens showed a much narrower intra-shell $\delta^{18}\text{O}$
510 variability compared to the modern ones (Fig. 12). By contrast the average shell $\delta^{13}\text{C}$ values were higher

511 in late Holocene specimens by ca. 1.3‰ compared to the modern shells. Their intra-shell $\delta^{13}\text{C}$ range was
512 also less variable and narrower than in modern specimens.

513 These isotopic differences likely reflect changes in water temperature and hydrological balance between
514 the late Holocene and present day at the LLS, although the magnitude of these changes remains
515 complex to estimate. For example, we have demonstrated that both temperature and $\delta^{18}\text{O}_w$ values
516 display strong seasonal variations in the LLS, and were both simultaneously responsible for intra-annual
517 shell $\delta^{18}\text{O}$ variability in modern specimens. Intra-annual ST and $\delta^{18}\text{O}_w$ values are unknown for the past,
518 and as such, the application of palaeotemperature equations on fossil shells from estuarine
519 environments are greatly limited (Ingram et al., 1996).

520 Despite these limitations, the lower variability in intra-shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values may reflect a reduced
521 seasonality in water temperature and/or salinity during the late Holocene compared to the present day.
522 It is worth noting that several lines of evidence indicate a general sea-level highstand along the southern
523 coast of Brazil during the middle Holocene, followed by a drop to the present day level (Angulo et al.,
524 1999; 2006). Recent geomorphological and sedimentary analyses reveal that during the early Holocene
525 the LLS, and surrounding areas, were submerged by the effect of post-glacial sea-level rise, forming a
526 large bay. At that time, the delta of the Tubarão River was retracted further inland, toward the Serra do
527 Tabuleiro. This palaeobay was subject to direct oceanic circulation. A change from this transgressive
528 open-marine embayment to a coastal lagoon occurred about 6 ka cal BP, due to the achievement of a
529 balance between sea-level rise and sedimentary supply, and the consequent formation of a sand barrier
530 to the south of Entrada da Barra inlet. Subsequently, the Tubarão river delta silted up most of this bay-
531 lagoon and the river inflow gradually advanced in Santo Antônio Lagoon until reaching its current
532 configuration (Giannini et al., 2010; Fornari et al., 2012). The intra-shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values discussed in
533 the present study would corroborate these palaeoenvironmental reconstructions. Archaeological shell
534 isotope data indirectly indicate a reduced seasonal freshwater-seawater balance at ca. 3 ka cal BP, which
535 agrees reasonably with the increased marine circulation. Modern shells instead show a higher seasonal
536 variability in freshwater-seawater balance due to the modern configuration of the LLS.

537 An additional factor that could have promoted the lower amplitudes of intra-shell $\delta^{18}\text{O}$ values may have
538 been a reduction in precipitation and/or changes in $\delta^{18}\text{O}$ values of precipitations. While the former
539 could have an indirect effect on the $\delta^{13}\text{C}$ by increasing $\delta^{13}\text{C}_{\text{DIC}}$ values, the latter would not explain the
540 reduced amplitude of intra-shell $\delta^{13}\text{C}$ values. Furthermore palaeoclimate reconstructions based on
541 speleothem $\delta^{18}\text{O}$ values and Sr/Ca ratios from southern Brazil point to an intense activity of the South
542 American Monsoon System during the late Holocene. This atmospheric system is the dominant
543 precipitation regime in southern Brazil and is responsible for the strong seasonal variation in rainfall
544 $\delta^{18}\text{O}$ values over the region (Cruz et al., 2005a,b; Bernal et al., 2016). Thus the most likely explanation is

545 that covariation in shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values and their reduced seasonal amplitude reflect a reduced
546 seasonal seawater-freshwater balance, and enhanced marine exposure.

547 Other factors might have contributed to the overall decrease of shell $\delta^{13}\text{C}$ values in modern specimens
548 (ca. 1.3‰) compared to those from late Holocene. In general, a positive shift of 1 ‰ to 1.5 ‰ is
549 expected in the $\delta^{13}\text{C}$ values of modern aquatic carbonates due to the increased emission of ^{12}C in the
550 atmosphere by industrial burning of fossil fuels (e.g. Friedli et al., 1986; Sonnerup et al., 1999; Surge et
551 al., 2003). The lower shell $\delta^{13}\text{C}$ values in modern specimens might also reflect a general decrease of
552 $\delta^{13}\text{C}_{\text{DIC}}$ values due to higher nutrient supply and eutrophication of Santo Antônio Lagoon (e.g. Surge et
553 al., 2003). For example, Barros et al. (2010) report the lowest $\delta^{13}\text{C}_{\text{DIC}}$ values in Babitonga Bay in areas
554 that receive heavy loads of untreated domestic sewage. This might be expected for Tubarão River today
555 as it drains areas affected by the use of pesticides, waste from intensive pig farming, as well as industrial
556 and urban effluents (Osório et al., 2014). Moreover, differing average shell $\delta^{13}\text{C}$ values between
557 archaeological and modern specimens could be associated with changes in vegetation composition and
558 structure in the LLS from the late Holocene. Decaying organic matter derived from plants contributes to
559 defining the $\delta^{13}\text{C}$ values of DIC (Mook and Tan, 1991). Present day vegetation at the LLS includes species
560 found mainly in salt marshes (e.g. *Spartina alterniflora*) and swamps (e.g. *Acrostichum aureum*),
561 together with small patches of mangrove forest (e.g. *Laguncularia racemosa*). However, the LLS marks
562 the present southern limit of the western South Atlantic mangroves, which are replaced southwards by
563 salt marsh vegetation (Schaeffer-Novelli et al., 1990; Soares et al., 2012). *Laguncularia racemosa*, the
564 dominant mangrove vegetation in the LLS, has a local $\delta^{13}\text{C}$ value typical of C3 plants (-26.4 ‰), while
565 local *Spartina alterniflora* from salt marshes has a $\delta^{13}\text{C}$ value consistent with C4 plants (-12.7 ‰)
566 (Tognella et al., 2016). Higher average shell $\delta^{13}\text{C}$ values in archaeological specimens could, to some
567 degree, reflect a higher contribution of salt marsh-derived C4 plant types to dissolved inorganic carbon
568 at 3 cal ka BP compared to present day.

569 How past populations interacted and responded to coastal and environmental changes at the LLS during
570 the Holocene is still a matter of debate. The sambaqui culture had its maximum expansion during the
571 middle-late Holocene (from 6 to 1.5 ka BP), as attested by the emergence and proliferation of shell
572 mounds, most of which are made of the shells of *A. flexuosa*. In agreement with other
573 palaeoenvironmental records, our results indicate that the builders of Cabeçuda shell mound at ca. 3 ka
574 BP exploited *A. flexuosa* in environments marked by lower salinity variations compared to present day at
575 the LLS. Given the limited tolerance of *A. flexuosa* to low salinity waters, the results could reinforce the
576 hypothesis that reorganization of coastal environments and increased oceanic exposure created more
577 suitable conditions for *A. flexuosa* in the study area between 6 to 1.5 ka BP, contributing to maintain a
578 long-term cultural practice.

579

580 **Conclusion**

581

582 The bivalve *Anomalocardia flexuosa* is a widely distributed intertidal and subtidal mollusc in coastal
583 areas of Latin America, from the Caribbean to Uruguay. It is abundant in archaeological and sedimentary
584 records, and constitutes an important economic resource for communities (both past and present) living
585 along the Brazilian coast. Using highly-resolved sclerochronological and stable isotopic analyses, we
586 unlocked relevant biological and environmental information from modern and sub-fossil mollusc shells
587 from the Laguna Estuarine System in southern Brazil. We demonstrated the effect of temperature and
588 salinity on the seasonal growth patterns in modern specimens from this subtropical coastal area of
589 Atlantic South America. The isotopically recorded information in the aragonitic shell carbonate thus can
590 be used to assess past environmental conditions using fossil shells from archaeological and sedimentary
591 records. Sclerochronological information (shell growth patterns and stable isotope) could potentially
592 help assessment and management strategies while revealing the impact of local environmental
593 conditions on modern populations.

594

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596

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615

616 **Figure captions**

617 Figure 1. Laguna Lagoonal System (LLS) in southern Brazil and the sample localities of instrumentally
618 recorded salinity and temperature data, and $\delta^{18}\text{O}_w$ values. Living specimens were collected at localities
619 12, 13, 14 and 15. Archaeological specimens from Cabeçuda shell mound refers to locality 12. Surface
620 salinity, temperature and $\delta^{18}\text{O}_w$ values were sampled to record the environmental gradient from
621 freshwater to seawater endmembers. Satellite imagery from USGS (EarthExplorer) and NASA (Shuttle
622 Radar Topography Mission).

623 Figure 2. A) Specimen AF-13-1 from locality 13; the dotted line represent the axis of maximum growth.
624 B) Polished cross-section with manually sampled carbonates from the outer layers for stable isotope
625 analyses. The pigmented areas are carotenoids. C-E) Details of polished cross-section immersed in
626 Mutvei's solution showing distinct periodic microgrowth increments with variable width during neap (N,
627 continuous line) and spring (S, dotted line) tides. The growth rate decreases with ontogenetic age, from
628 the umbo toward the ventral margin. Dog: direction of growth.

629 Figure 3. Optical microscopy images of the internal part of the *Anomalocardia flexuosa* shell (AF-13-2)
630 obtained using a x10 objective. Regions labelled A-K were investigated using Raman spectroscopy.

631 Figure 4. Environmental information for the southern sector of the LLS, at Santo Antônio Lagoon. A)
632 Daily ST; B) SS; C) monthly total precipitation; D) daily average astronomical tidal oscillation; E) predicted
633 $\delta^{18}\text{O}_w$ values; F) estimated $\delta^{13}\text{C}_{\text{DIC}}$ values; and G) Chlorophyll *a* concentration. (A, B, E, F 205-point
634 moving average; D 2-point moving average).

635 Figure 5. A) Spatial and seasonal variation in SS and B) $\delta^{18}\text{O}_w$ values for the LLS.

636 Figure 6. $\delta^{18}\text{O}_w$ -SS relationship for the LLS over a period of one year.

637 Figure 7. Raman spectra acquired with 1cm^{-1} spectral resolution from different regions of the
638 *Anomalocardia flexuosa* shell (AF-13-2) measured at positions A-K in Fig. 6. Peaks denoted with *
639 correspond to the carotenoid signature, while peaks at 150, 204, 700 and 1081 cm^{-1} correspond to the
640 aragonite polymorph of calcium carbonate (as per Urmos et al., 1991). The prominent ν_3 and ν_1
641 carotenoid peaks are labelled. The ν_1 carotenoid peak shifts to lower wavenumbers as a function of
642 increasing pigmentation.

643 Figure 8. An example of the growth pattern in *Anomalocardia flexuosa* (specimen AF-13-1). A) Increment
644 width (including 10-days moving average) and B) detrended growth increments using exponential fit
645 (ratio-based GI).

646 Figure 9. Filtered detrended time series using A) high pass, B) low pass and C) band pass filters. D)
647 Alignment between band pass filtered data and average daily tidal oscillation (8-points moving average)
648 for the last 200 days before shell collection. Wavelet power spectrum of detrended microgrowth
649 increments of living specimen sampled at Santo Antônio Lagoon. The power in the wavelet power
650 spectrum has been scaled by the global wavelet spectrum. The cross-hatched region is the cone of
651 influence, where zero padding has reduced the variance. Black contour is the 95% significance level,
652 using a red-noise (autoregressive lag1) background spectrum.

653 Figure 10. Shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values (2-point moving average) and detrended shell growth increments
654 (10-point moving average) of modern *Anomalocardia flexuosa*. The vertical dotted lines represent the
655 ontogenetic years. The red dotted line in specimen AF-13-2 marks the visible traces of carotenoids.

656 Figure. 11. Temporal alignment between measured and predicted (250-point moving average) shell $\delta^{18}\text{O}$
657 values for the last years of shell formation (years 2008-2009) in four specimens. The shell $\delta^{18}\text{O}$ values
658 were arranged so that they match the predicted values as closely as possible.

659 Figure 12. Shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of late Holocene archaeological specimens from Cabeçuda shell
660 mound. The vertical dotted lines in CAB3 mark the external growth checks observed only on this
661 specimen. The red and grey bands represent the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variability, respectively, observed in
662 modern specimens.

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