Using stable carbon isotopes to quantify radiocarbon reservoir age offsets in the coastal Black Sea

Guillaume Soulet[1†,*], Liviu Giosan[1], Clément Flaux[2], Valier Galy[3]

[1]Department of Geology and Geophysics, Woods Hole Oceanographic Institution, 266 Woods Hole Road, Woods Hole, MA 02543, USA

[2]Centre National de la Recherche Scientifique EcoLab (Laboratoire d'Ecologie Fonctionnelle et Environnement), Université Paul Sabatier, Toulouse, France

[3]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, 266 Woods Hole Road, MA 02543, USA

*Corresponding author. E-mail address: guillaume.s.soulet@durham.ac.uk, Tel. +44 191 33 41957

[†]Current address: Department of Geography, Durham University, South Road, Durham DH1 3LE, United Kingdom

ABSTRACT

Constraining radiocarbon reservoir age offsets is critical to deriving accurate calendar-age chronologies from radiocarbon (^{14}C) dating of materials which did not draw carbon directly from the atmosphere. The application of ^{14}C dating to such materials is severely limited in hydrologically sensitive environments like the Black Sea because of the difficulty to quantify reservoir age offsets, which can vary quickly and significantly through time, due to the dynamics of the biogeochemical cycling of carbon. Here we reconstruct radiocarbon reservoir age offsets ($R_{shell-atm}$) of Holocene bivalve shells from the coastal Black Sea relatively to their contemporaneous atmosphere. We show that the radiocarbon reservoir age offset and the stable carbon isotope composition of bivalve shells are linearly correlated in this region. From a biogeochemical standpoint, this suggests that inorganic stable carbon isotope and radiocarbon compositions of Black Sea coastal waters are controlled by the balance between autochthonous primary productivity and heterotrophic respiration of allochthonous pre-aged terrestrial organic matter supplied by rivers. This provided an important implication for Black Sea geochronology as the reservoir age offset of ^{14}C-dated bivalve shell can be inferred from its stable carbon isotope composition. Our results provide a fundamental and inexpensive geochemical tool which will considerably improve the accuracy of Holocene calendar age chronologies in the Black Sea.

INTRODUCTION

The radiocarbon reservoir age offset (R) of an organism is the difference between its ^{14}C age and that of the atmospheric CO_2 at the time this organism was alive (Stuiver and Polach, 1977; Ascough et al., 2005; Jull et al., 2013; Soulet et al., 2016). Radiocarbon reservoir age offsets are in "^{14}C years". Very importantly, any ^{14}C age obtained from an organism that did not incorporate its carbon directly from the atmosphere must be corrected for a reservoir age offset to provide an accurate estimate of its calendar age using the atmospheric radiocarbon calibration curve (Reimer et al., 2013). These corrections are crucial as they can range from a few hundreds to thousands of ^{14}C years (Siani et al., 2000, 2001; Bondevik et al., 2006; Kuzmin et al., 2007; Soulet et al., 2011a).

Recent studies suggested that some geochemical characteristics of an organism could be related to its reservoir age offset. For instance, it has been shown that the radiocarbon reservoir age offset of modern Baltic Sea *Macoma* bivalve shell was inversely related to its shell ^{87}Sr/^{86}Sr ratio (Lougheed et al., 2016). Accurately determining the calendar age of ^{14}C-dated archeological remains of human populations that draw their carbon from various sources as a result of mixed diet is challenging. For example, individuals from a population that mostly eats fish can have their stable carbon and nitrogen compositions impacted (Schoeninger et al., 1983; Schoeninger and Deniro, 1984) and even exhibit a reservoir age offset (Dewar and Pfeiffer, 2010; Olsen et al., 2010; Ascough et al., 2012; Wood et al., 2013). It has been shown from two archeological sites from the region of Lake Baikal that the radiocarbon reservoir age offset of human bones was linearly correlated to their nitrogen isotopic composition (δ^{15}N) (Schulting et al., 2014), and for one site to both their δ^{15}N and stable carbon isotopic compositions (δ^{13}C) (Bronk Ramsey et al., 2014; Schulting et al., 2014). Another study showed that radiocarbon reservoir age offset of human bones from a medieval Icelandic cemetery can be inferred using the nitrogen, carbon and sulfur isotopic compositions of the bones (Sayle et al., 2016). These studies are fundamental in the field of geochronology because they showed that it is possible to untangle the very complicated reservoir age offset correction issue.

The Black Sea is currently connected to the global oceans solely via the narrow and shallow Bosporus Strait (Fig. 1), leading to restricted water exchanges with the Mediterranean Sea and permanent stratification (Özsoy and Ünlüata, 1997). During the last glacial lowstand and for much of the deglacial sea-level rise, the Mediterranean connection was closed and the

Black Sea was a large lake (e.g., Ross et al., 1970; Badertscher et al., 2011). The understanding of the glacial-deglacial hydrologic changes of the Black Sea has been recently refined (Major et al., 2006; Bahr et al., 2006; Kwiecien et al., 2009; Soulet et al., 2011a, 2013), as well as of its Holocene evolution (e.g., van der Meer et al., 2008; Giosan et al., 2012; Coolen et al., 2013). However, a robust chronological framework for the Holocene Black Sea is still lacking, thereby limiting interpretation and still precluding an understanding of the sequence of events that led to the reconnection of the Black Sea to the Mediterranean Sea that occurred sometime during the early Holocene (Ryan et al., 1997; Aksu et al., 2002; Major et al., 2006; Giosan et al., 2009; Soulet et al., 2011b; Yanko-Hombach et al., 2014). The uncertainty surrounding the timing of the reconnection – and by extension, the Black Sea chronological framework – is related to unconstrained radiocarbon reservoir age offset correction. This weakness has long been recognized (Jones and Gagnon, 1994; Giosan et al., 2009; Kwiecien et al., 2008; Soulet et al., 2011a) but it has never been fully solved.

Here, we reconstruct radiocarbon reservoir age offsets of bivalve shells from the coastal Black Sea and relate them to their $\delta^{13}C$ values, in an attempt to define radiocarbon reservoir ages on the basis of the relationship, with the potential to considerably improve the chronological framework of the Black Sea sediment archives.

METHODS AND SAMPLES

Reconstructions of radiocarbon reservoir age offsets typically rely upon ^{14}C determinations on terrestrial/marine(or lacustrine) pairs or multiple pairs (Ascough et al., 2005; Jull et al., 2013; Soulet, 2015; Soulet et al., 2016). From cores collected in the western coastal Black Sea (Fig. 1), five single pairs of articulated bivalve shells and pieces of terrestrially-derived plant material were sampled from the same sediment layers in order to ensure contemporaneous deposition, and their ^{14}C ages and stable carbon isotope values were determined.

Radiocarbon and stable carbon isotope measurements

As a pre-cleaning, the bivalve shells were sonicated and rinsed in deionized water at least 10 times. Then the shell carbonate was converted into CO_2 following National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility's standard phosphoric acid hydrolysis procedure (McNichol et al., 1994). The analyzed terrestrial plant materials were subjected to NOSAMS standard acid-base-acid pre-treatment and converted to CO_2 through the sealed tube combustion method (McNichol et al., 1994). The CO_2 was then converted to graphite and analyzed for its ^{14}C composition by Accelerator Mass Spectrometry (AMS) at NOSAMS. Two shell samples and two terrestrial plant material samples were prepared and graphitized at Centre de Datation par le RadioCarbone (CDRC, Lyon, France) following procedures similar to those performed at NOSAMS and measured by AMS at the Laboratoire de Mesure du Radiocarbone (LMC14-ARTEMIS, Saclay, France). Results are corrected for the $^{13}C/^{12}C$ ratio as measured on the AMS (Santos et al., 2007) and are reported in Fm notation. Fm is identical to the A_{SN}/A_{ON} metric (Stuiver and Polach, 1977), the $^{14}a_N$ notation (Mook and van der Plicht, 1999), and to the $F^{14}C$ notation (Reimer et al., 2004). Corresponding conventional ^{14}C ages (ρ) reported in ^{14}C years Before Present (AD 1950) were calculated according to:

$$\rho = -8033 \ln(\text{Fm}) \tag{1}$$

The stable carbon isotope values of the dated samples were obtained from a 10%-aliquot taken out of the produced CO_2 using an Optima or VG Prism series II stable Isotope Ratio Mass Spectrometer (IRMS) at NOSAMS. $^{13}C/^{12}C$ ratios are reported in the $\delta^{13}C$ notation (‰ relative to Vienna Pee Dee Belemnite, or VPDB).

Reservoir age offset calculation

Radiocarbon reservoir age offsets of the bivalve shells, relative to their contemporaneous atmosphere ($R_{shell-atm}$), were calculated based on the ^{14}C ages of the paired bivalve shell (ρ_{shell}) and terrestrially-derived plant material (ρ_{plant}) (e.g., Ascough et al., 2005; Jull et al., 2013; Soulet et al., 2016):

$$R_{shell-atm} = \rho_{shell} - \rho_{plant} \quad (2)$$

The ^{14}C ages of the terrestrially-derived plant material are assumed to correspond to the ^{14}C age of the atmosphere at the time the bivalves were living. Five $R_{shell-atm}$ values were calculated according to this method, based on the following paired samples of Black Sea shells and terrestrially-derived plant materials found co-located in the same sediment layer:

i) A pair of an articulated juvenile *Dreissena sp.* bivalve and a sample of fragile foliar material, picked from a peat where the bivalve was found embedded and dated to 10,600–11,080 (95%) cal yr BP (core SG in the modern Danube delta). The bivalve, being a juvenile, was small and could have easily been transported onto the marsh surface where the peat was developing.

ii) A pair of a *Monodacna caspia* bivalve (single valve) and a fragile piece of *Phragmites* reed dated to 9,040–9,420 (95%) cal yr BP (core MD04-2774; offshore the modern Danube delta).

iii) Two paired samples of an articulated *Mytilus galloprovincialis* bivalve and charcoal from a ~15cm-thick archeological layer recovered in several cores (SOZ-7 cores; Alepu lagoon, Bulgaria) (Flaux et al., 2016). The archeological site, dated to 5,050–5,300 (95%) cal yr BP (beginning of Early Bronze Age), was a pile-dwelling settlement recently discovered in the present Alepu lagoon (Flaux et al., 2016). The piles were 1 m long and 5 to

6 cm diameter and are made of oak. The bark was still in place showing that the wood was used directly after felling. The small diameter of the piles suggest that they were less than 40 years old at felling (Flaux et al., 2016). The piles indicate the beginning of the pile-dwelling occupation and were dated to 4550 ± 30 ^{14}C yr BP. The charcoal pieces that we used to calculate the reservoir age offsets were found in the archeological layer of the site occupation that lasted less than 80 years (Flaux et al., 2016). The two charcoal samples used were dated to 4525 ± 30 and 4475 ± 30 ^{14}C yr BP. These ^{14}C ages are not significantly different from those of the piles (Flaux et al., 2016). This suggests that the charcoals must originate from wood that lived during the occupation, and thus characterized by very little old wood effect, if any.

iv) A pair of an articulated *Dreissena polymorpha* bivalve and a sample of small fragments of twigs dated to 4,540–4,820 (95%) cal yr BP (core NE-1 in the inner Danube delta).

One additional $R_{shell-atm}$ value was obtained from the calendar age of a mussel (*Mytilus galloprovincialis*) collected alive in 1931 (offshore of west Crimea) (Jones and Gagnon, 1994). In this case, the ^{14}C composition of the atmosphere in 1931 was obtained from linear interpolation of the radiocarbon calibration curve IntCal13 (Reimer et al., 2013), then the $R_{shell-atm}$ value was calculated according to the above equations (substituting the subscript "plant" by "IntCal13"). The radiocarbon reservoir age offset of two bivalve shells collected alive during the 19th century in the Black Sea are reported in Siani et al. (2000) but stable carbon isotopes were not published, and thus cannot be included in this study.

All radiocarbon and reservoir age offset data are compiled in the supplementary material (Table S1).

RESULTS AND DISCUSSION

Calculated $R_{shell-atm}$ values ranged widely between 340 and 1,100 ^{14}C years. This finding demonstrates that reservoir age offsets varied substantially in Black Sea coastal environments during the Holocene. The $\delta^{13}C_{shell}$ values also ranged widely between –8 to +1 ‰ VPDB. We also found that the $R_{shell-atm}$ values are linearly correlated to the $\delta^{13}C_{shell}$ values (r^2=0.87; p-value < 0.01; n=6) (Fig. 2):

$$R_{shell-atm} = 473(\pm 58) - 68(\pm 13) \times \delta^{13}C_{shell} \quad (3)$$

Biogeochemical significance of the $R_{shell-atm}$-$\delta^{13}C_{shell}$ line

In order to understand the biogeochemical implications of the line, we explore the potential ^{14}C-^{13}C end-members of the coastal Black Sea. Here, we use the isotopic form of the reservoir age offset. This metric called the $\delta^{14}R$ value (Soulet et al., 2016) is calculated based on the exact same ^{14}C measurements used to calculate $R_{shell-atm}$:

$$\delta^{14}R_{shell-atm} = 1000\left(\frac{Fm_{shell}}{Fm_{plant}} - 1\right)‰ \quad (4)$$

The link between $\delta^{14}R$ and R is straightforward:

$$R_{shell-atm} = -8033\ln\left(\frac{Fm_{shell}}{Fm_{plant}}\right) \quad (5)$$

The $\delta^{14}R$-$\delta^{13}C$ relationship (Fig. 3) is also a line (r^2=0.87; p-value < 0.01; n=6):

$$\delta^{14}R_{shell-atm} = -57.3(\pm 6.6) + 7.8(\pm 1.5) \times \delta^{13}C_{shell} \quad (6)$$

Over the observed range of $\delta^{13}C$ values (–8 to +1 ‰ VPDB), the difference in the $R_{shell-atm}$ inferred from Eq. 3 or Eq. 6 is minimal (<10 ^{14}C years). Instead, for very negative $\delta^{13}C$ values the difference in calculated $R_{shell-atm}$ can be up to thousands of ^{14}C years. This is because $R_{shell-atm}$ is a logarithmic function of the Fm values. This is why we must use the $\delta^{14}R$-$\delta^{13}C$ relationship to understand the biogeochemical processes explaining the line.

Bivalves form their carbonate shell primarily from the dissolved inorganic carbon (DIC) component of the water column, over several months to a few years as longevity of the bivalves studied here (*Mytilus galloprovinciallis* and *Dreissena rostriformis*) is ~10 years and probably less (Karatayev et al., 2006; Okaniwa et al., 2010). Thus, the shell isotopic composition reflects an integrated carbon isotopic composition of the DIC of the water in which they formed (Leng and Marshall, 2004). The $\delta^{14}R_{shell-atm}$-$\delta^{13}C_{shell}$ line (Eq. 6) indicates a ^{13}C enrichment of the water DIC when the water DIC ^{14}C composition becomes closer to that of the atmosphere (i.e., $\delta^{14}R_{shell-atm}$ becomes closer to 0‰). In other words, the ^{14}C composition of the water DIC becomes equilibrated with that of the atmosphere, when its stable isotope composition becomes enriched in ^{13}C. The enrichment in ^{13}C may also result in DIC stable carbon isotope composition equilibration with that of the atmosphere but with a fractionation in $\delta^{13}C$ from atmospheric CO_2 to bicarbonate of 7 to 8‰ (Mook et al., 1974; Romanek et al., 1992).

The trend of the line suggests that the composition of the DIC of Black Sea coastal waters may have been driven mainly by the balance between autochthonous primary productivity and heterotrophic respiration of ^{14}C-depleted (pre-aged) allochthonous terrestrial organic matters supplied by rivers. Indeed, the increased surface primary productivity during phytoplankton photosynthesis leads to ^{13}C enrichment in the DIC (Hollander and McKenzie, 1991; Leng and Marshall, 2004). This also leads to increased transfer of atmospheric CO_2 to surface water via green algae and phytoplankton demand for CO_2 (Deuser, 1970; Hollander and McKenzie, 1991; Li et al., 2017), and may result in an equilibration of the surface water ^{14}C composition with that of the atmosphere, meaning that $\delta^{14}R_{shell-atm}$ tends towards 0‰. The latter is supported by ^{14}C activity measurements of the DIC of Black Sea coastal waters in May 2004 showing that it had a similar ^{14}C activity to that of the atmosphere on the day of sampling (Fontugne et al., 2009), with $\delta^{14}R$ values of up to –4‰ (equivalent to a reservoir age offset of only ~30 ^{14}C years), i.e, very close to 0 ‰. It is also supported by the presence of bomb ^{14}C in short chain saturated fatty acids and alkenones – both produced by phytoplankton – extracted from core top sediments collected in the early 2000s from the coastal Black Sea (Kusch et al., 2010, 2016).

The primary productivity vs. heterotrophic respiration balance hypothesis is further supported by the composition of the end-members defining the $\delta^{14}R_{shell\text{-}atm}$-$\delta^{13}C_{shell}$ linear relationship. Indeed, assuming a complete productivity-driven equilibration of the surface water with the atmosphere, i.e., $\delta^{14}R_{shell\text{-}atm}$ = 0‰, the line predicts a $\delta^{13}C_{shell}$ value of 6.0 ± 1.9‰ VPDB for the carbonate phase (i.e., the mineral phase of the shell). At a range of temperatures typical for Black Sea surface waters, the $\delta^{13}C$ of the carbonate phase is enriched by ~11-13‰ compared to CO_2 (Romanek et al., 1992). Thus, the $\delta^{13}C$ value of a carbonate derived from atmospheric CO_2 with a pre-industrial $\delta^{13}CO_2$ value of –6.4‰ with respect to VPDB (Schmitt et al., 2012) would be of 4.5 to 6.5‰ VPDB. This range is in excellent agreement with the shell $\delta^{13}C$ value of 6.0 ± 1.9‰ VPDB, predicted by the line in the case of CO_2 equilibration of the surface water with the atmosphere (Fig. 3). In addition, in the case of no autochthonous productivity at all, the organic matter utilized during heterotrophic respiration would have $\delta^{13}C$ values of –25 to –27‰ VPDB, typical for Black Sea terrestrial organic matter (Kusch et al., 2010). Carbonate $\delta^{13}C$ originating from pure respiration of terrestrial organic matter would thus range around –12 to –16‰ VPDB (Romanek et al., 1992), corresponding to predicted $\delta^{14}R_{shell\text{-}atm}$ values of –150 to –180‰. These latter values are compatible with surface sediment $\delta^{14}R_{sed\text{-}atm}$ of –200‰, calculated from total organic carbon ^{14}C ages offshore of the Danube River (Kusch et al., 2010) (Fig. 3). Mineralization of pre-aged terrestrial organic matter by microbial respiration occurs in temperate lakes and streams of Quebec (McCallister and del Giorgio, 2012). Our results suggest that pre-aged terrestrial organic matter as old as ~1500 ^{14}C years supplied by rivers is also converted to CO_2 by heterotrophic bacteria in the Black Sea coastal waters.

A hard water effect (HWE) origin, i.e., reservoir age offset mainly explained by the contribution of ^{14}C-depleted riverine DIC from the dissolution of outcropping carbonates, seems unlikely. Indeed, carbonate minerals can be weathered through two main pathways. The carbonic acid pathway:

$$CaCO_3 + CO_{2,g} + H_2O \rightarrow Ca^{2+} + 2HCO_3^- \quad (7)$$

This pathway involves $CaCO_3$ and atmospheric CO_2 with $\delta^{14}R$ values of –1000 and 0‰ respectively, and $\delta^{13}C$ values of ~0 and –6.5‰ VPDB (pre-industrial). Thus, the carbonate derived from the resulting DIC (~HCO_3^-) would have a $\delta^{13}C$ value of –2 to 0‰ VPDB (Romanek et al., 1992) and $\delta^{14}R_{HWE\text{-}atm}$ of –500‰ (Fig. 3).

The second carbonate weathering pathway involves sulfuric acid, itself produced by the oxidation of sulfide minerals like pyrite (Calmels et al., 2007):

$$2CaCO_3 + H_2SO_4 \rightarrow 2Ca^{2+} + 2HCO_3^- + SO_4^{2-} \quad (8)$$

In this case the carbonate derived from the resulting DIC would have a $\delta^{13}C$ value of ~0‰ VPDB and $\delta^{14}R_{HWE\text{-}atm}$ would be of –1000‰. None of these endmembers fits the coastal Black Sea $\delta^{14}R$-$\delta^{13}C$ line (Fig. 3).

Oxidation of old methane could be another explanation, as it seems to impact on the ^{14}C composition of DIC of the present day Black Sea waters in the deeper part of the basin (slope and deep basin) (Fontugne et al., 2009). However, for very negative $\delta^{13}C$ values typical of methane oxidation (e.g. –60‰; Kessler et al., 2006), the extension of the line predicts a $\delta^{14}R_{shell\text{-}atm}$ of –400 to –450‰, in disagreement with $\delta^{14}R$ values for Black Sea methane being as low as –850‰ (Kessler et al., 2006). Thus, oxidation of methane is unlikely to explain the reservoir age offset in the coastal settings of the western Black Sea.

Improving Black Sea geochronology

The coastal Black Sea $R_{shell-atm}$-$\delta^{13}C_{shell}$ line (Eq. 3; Fig. 2) has fundamental implications for the Black Sea geochronology. It is now possible to use bivalve shell $\delta^{13}C$ values as a proxy for reconstructing the reservoir age offset in the coastal Black Sea. Moreover, this $\delta^{13}C$-based tool can be applied on the same bivalve shell that is being ^{14}C dated, leading to a customized reservoir age offset correction, which is crucial to providing an accurate estimate of the calendar age for the ^{14}C-dated shell. Using this proxy is inexpensive since most ^{14}C laboratories can provide the $\delta^{13}C$ values of the dated material. One should note that the $\delta^{13}C$ determination must be performed by IRMS on the CO_2 produced from the dated material and reported vs. VPDB. The $\delta^{13}C$ value measured on the AMS during radiocarbon measurement should not be used because of potential large instrumental fractionation effects (Santos et al., 2007).

At this stage of the research, i) any vital effect on the $\delta^{13}C$ value seems of second order importance, but the use of the $R_{shell-atm}$-$\delta^{13}C_{shell}$ line should be restricted to bivalve shells and for $\delta^{13}C$ values of 2 to –10‰; ii) the application of the line should be restricted to the Holocene, in coastal to near coastal settings of the western Black Sea; iii) the line applies for both marine and lacustrine periods of the basin evolution – suggesting that the composition of the endmembers did not change much through the Holocene and that the pathway of terrestrial carbon mineralization (from Black Sea lacustrine or marine biotic communities) is also of second order importance.

CONCLUSION

Our results provide new pieces of information about carbon cycling in the coastal Black Sea. They suggest that the balance between primary productivity and heterotrophic respiration of terrestrial carbon supplied by rivers is governing the carbon isotopic composition of the

Black Sea coastal waters since early Holocene. As a consequence, they provide an invaluable $\delta^{13}C$-based tool to quantify reservoir age offset for the Holocene Black Sea coastal settings. This will undoubtedly help refine our understanding of the hotly debated last reconnection of the Black Sea and more generally provide constrained calendar age-depth models to the numerous studied sediment archives of the Black Sea. Other basins, e.g., the Caspian Sea, the Aral Sea or large lakes, may potentially yield such correlation between the ^{14}C reservoir age offset and $\delta^{13}C$ value of shells and deserve future investigation. This study, in line with other pioneering studies (Bronk Ramsey et al., 2014; Lougheed et al., 2016; Schulting et al., 2014), shows that correlations between the reservoir age offset and another geochemical parameter from the same material must be sought, with the aim of untangling the problem of reservoir age offset when deriving calendar age chronologies from non-atmospheric ^{14}C measurements.

ACKNOWLEDGEMENTS

G.S. acknowledges the Postdoctoral Scholar Program at the Woods Hole Oceanographic Institution (WHOI) with funding provided by the NOSAMS facility (OCE-1239667). He thanks the entire NOSAMS crew with special thanks to Ann McNichol and William Jenkins, as well as Mary Lardie, Liz Klein and Al Gagnon for assistance in the lab. L.G. thanks Florin Filip and Stefan Constantinescu for help in collecting Danube delta cores as well as to WHOI for continuous support to his work in the Danube delta and the Black Sea. We thank Gilles Lericolais from Ifremer for providing access to core MD04-2774. This work is a contribution to the ASSEMBLAGE project funded by the European Commission (EVK3-CT-2002-00090).

REFERENCES CITED

Aksu AE, Hiscott RN, Mudie PJ, Rochon A, Kaminski MA, Abrajano T, Yaşar D. 2002. Persistent Holocene outflow from the Black Sea to the Eastern Mediterranean

Contradicts Noah's Flood Hypothesis. *GSA Today* 12(5):4–10.

Ascough PL, Cook GT, Dugmore AJ. 2005. Methodological approaches to determining the marine radiocarbon reservoir effect. *Progress in Physical Geography* 29(4):532–547.

Ascough PL, Church MJ, Cook GT, Dunbar E, Gestsdóttir H, McGovern TH, Dugmore AJ, Fridriksson A, Edwards KJ. 2012. Radiocarbon reservoir effects in human bone collagen from northern Iceland. *Journal of Archaeological Science* 39(7):2261–2271.

Badertscher S, Fleitmann D, Cheng H, Edwards RL, Göktürk OM, Zumbühl A, Leuenberger M, Tüysüz O. 2011. Pleistocene water intrusions from the Mediterranean and Caspian seas into the Black Sea. *Nature Geoscience* 4(4):236–239.

Bahr A, Arz HW, Lamy F, Wefer G. 2006. Late glacial to Holocene paleoenvironmental evolution of the Black Sea, reconstructed with stable oxygen isotope records obtained on ostracod shells. *Earth and Planetary Science Letters* 241(3–4):863–875.

Bondevik S, Mangerud J, Birks HH, Gulliksen S, Reimer PJ. 2006. Changes in North Atlantic Radiocarbon Reservoir Ages During the Allerod and Younger Dryas. *Science* 312(5779):1514–1517.

Bronk Ramsey C, Schulting R, Goriunova OI, Bazaliiskii VI, Weber AW. 2014. Analyzing radiocarbon reservoir offsets through stable nitrogen isotopes and Bayesian modeling: A case study using paired human and faunal remains from the Cis-Baikal region, Siberia. *Radiocarbon* 56(2):789–799.

Calmels D, Gaillardet J, Brenot A, France-Lanord C. 2007. Sustained sulfide oxidation by physical erosion processes in the Mackenzie River basin: Climatic perspectives. *Geology* 35(11):1003.

Coolen MJL, Orsi WD, Balkema C, Quince C, Harris K, Sylva SP, Filipova-Marinova M, Giosan L. 2013. Evolution of the plankton paleome in the Black Sea from the Deglacial to Anthropocene. *Proceedings of the National Academy of Sciences* 110(21):8609–8614.

Deuser WG. 1970. Isotopic evidence for diminishing supply of available carbon during diatom bloom in the Black Sea. *Nature* 225(5237):1069–1071.

Dewar G, Pfeiffer S. 2010. Approaches to estimating marine protein in human collagen for radiocarbon date calibration. *Radiocarbon* 52(4):1611–1625.

Flaux C, Rouchet P, Popova T, Sternberg M, Guibal F, Talon B, Baralis A, Panayotova K, Morhange C, Riapov AV. 2016. An Early Bronze Age pile-dwelling settlement discovered in Alepu lagoon (municipality of Sozopol, department of Burgas), Bulgaria. *Méditerranée* 126:57–70.

Fontugne M, Guichard F, Bentaleb I, Strechie C, Lericolais G. 2009. Variations in ^{14}C reservoir ages of Black Sea waters and sedimentary organic carbon during anoxic periods: Influence of photosynthetic versus chemoautotrophic production. *Radiocarbon* 51(3):969–976.

Giosan L, Coolen MJL, Kaplan JO, Constantinescu S, Filip F, Filipova-Marinova M, Kettner AJ, Thom N. 2012. Early anthropogenic transformation of the Danube-Black Sea system. *Scientific Reports* 2(1):582.

Giosan L, Filip F, Constatinescu S. 2009. Was the Black Sea catastrophically flooded in the early Holocene? *Quaternary Science Reviews*. 28(1–2):1–6.

Hollander DJ, McKenzie JA. 1991. CO_2 control on carbon-isotope fractionation during aqueous photosynthesis: A paleo-pCO_2 barometer. *Geology* 19(9):929–932.

Jones GA, Gagnon AR. 1994. Radiocarbon chronology of Black Sea sediments. *Deep Sea*

Research Part I: Oceanographic Research Papers* 41(3):531–557.

Jull AJT, Burr GS, Hodgins GWL. 2013. Radiocarbon dating, reservoir effects, and calibration. *Quaternary International*. 299:64–71.

Karatayev AY, Burlakova LE, Padilla DK. 2006. Growth rate and longevity of Dreissena polymorpha (Pallas): A review and recommendations for future study. *Journal of Shellfish Research* 25(1):23–32.

Kessler JD, Reeburgh WS, Southon J, Seifert R, Michaelis W, Tyler SC. 2006. Basin-wide estimates of the input of methane from seeps and clathrates to the Black Sea. *Earth and Planetary Science Letters* 243(3–4):366–375.

Kusch S, Rethemeyer J, Hopmans EC, Wacker L, Mollenhauer G. 2016. Factors influencing ^{14}C concentrations of algal and archaeal lipids and their associated sea surface temperature proxies in the Black Sea. *Geochimica et Cosmochimica Acta* 188:35–57.

Kusch S, Rethemeyer J, Schefuß E, Mollenhauer G. 2010. Controls on the age of vascular plant biomarkers in Black Sea sediments. *Geochimica et Cosmochimica Acta* 74(24):7031–7047.

Kuzmin YV, Nevesskaya LA, Krivonogov SK, Burr GS. 2007. Apparent ^{14}C ages of the “pre-bomb” shells and correction values (R, ΔR) for Caspian and Aral Seas (Central Asia). *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 259(1):463–466.

Kwiecien O, Arz HW, Lamy F, Plessen B, Bahr A, Haug GH. 2009. North Atlantic control on precipitation pattern in the eastern Mediterranean/Black Sea region during the last glacial. *Quaternary Research* 71(3):375–384.

Kwiecien O, Arz HW, Lamy F, Wulf S, Bahr A, Röhl U, Haug GH. 2008. Estimated reservoir ages of the Black Sea since the Last Glacial. *Radiocarbon* 50(1):99–118.

Leng MJ, Marshall JD. 2004. Palaeoclimate interpretation of stable isotope data from lake sediment archives. *Quaternary Science Reviews* 23(7–8):811–831.

Li Y, Qiang M, Jin Y, Liu L, Zhou A, Zhang J. 2017. Influence of aquatic plant photosynthesis on the reservoir effect of Genggahai Lake, Northeastern Qinghai-Tibetan Plateau. *Radiocarbon* in press doi:10.1017/RDC.2017.127.

Lougheed BC, van der Lubbe HJL, Davies GR. 2016. $^{87}Sr/^{86}Sr$ as a quantitative geochemical proxy for ^{14}C reservoir age in dynamic, brackish waters: Assessing applicability and quantifying uncertainties. *Geophysical Research Letters* 43(2):735–742.

Major CO, Goldstein SL, Ryan WBF, Lericolais G, Piotrowski AM, Hajdas I. 2006. The co-evolution of Black Sea level and composition through the last deglaciation and its paleoclimatic significance. *Quaternary Science Reviews* 25(17–18):2031–2047.

McCallister SL, del Giorgio PA. 2012. Evidence for the respiration of ancient terrestrial organic C in northern temperate lakes and streams. *Proceedings of the National Academy of Sciences* 109(42):16963–16968.

McNichol AP, Osborne EA, Gagnon AR, Fry B, Jones GA. 1994. TIC, TOC, DIC, DOC, PIC, POC — unique aspects in the preparation of oceanographic samples for ^{14}C-AMS. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 92(1–4):162–165.

Mook WG, Bommerson JC, Staverman WH. 1974. Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. *Earth and Planetary Science Letters* 22(2):169–176.

Mook WG, van der Plicht J. 1999. Reporting ^{14}C activities and concentrations. *Radiocarbon*

41(3):227–239.

Okaniwa N, Miyaji T, Sasaki T, Tanabe K. 2010. Shell growth and reproductive cycle of the Mediterranean mussel Mytilus galloprovincialis in Tokyo Bay, Japan: Relationship with environmental conditions. *Plankton and Benthos Research* 5(Supplement): 214–220.

Olsen J, Heinemeier J, Lübke H, Lüth F, Terberger T. 2010. Dietary habits and freshwater reservoir effects in bones from a Neolithic NE German cemetery. *Radiocarbon* 52(2):635–644.

Özsoy E, Ünlüata Ü. 1997. Oceanography of the Black Sea: A review of some recent results. *Earth-Science Reviews* 42(4):231–272.

Reimer PJ, Bard E, Bayliss A, Beck JW, Blackwell PG, Bronk Ramsey C., Buck CE, Cheng H, Edwards RL, Friedrich M, Grootes PM, Guilderson TP, Haflidason H, Hajdas I, Hatté C, Heaton TJ, Hoffmann DL, Hogg AG, Hughen KA, Kaiser KF, Kromer B, Manning SW, Niu M, Reimer RW, Richards DA, Scott EM, Southon JR, Staff RA, Turney CSM, van der Plicht J. 2013. IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 Years cal BP. *Radiocarbon* 55(4):1869–1887.

Reimer PJ, Brown TA, Reimer RW. 2004. Discussion: Reporting and calibration of post-bomb ^{14}C data. *Radiocarbon* 46(3):1299–1304.

Romanek CS, Grossman EL, Morse JW. 1992. Carbon isotopic fractionation in synthetic aragonite and calcite: Effects of temperature and precipitation rate. *Geochimica et Cosmochimica Acta* 56(1):419–430.

Ross DA, Degens ET, MacIlvaine J. 1970. Black Sea: Recent Sedimentary History. *Science* 170(3954):163–165.

Ryan WBF, Pitman WC, Major CO, Shimkus K, Moscalenko V, Jones GA, Dimitrov P, Gorür N, Sakinç M, Yüce H. 1997. An abrupt drowning of the Black Sea shelf. *Marine Geology* 138(1–2):119–126.

Santos GM, Southon JR, Griffin S, Beaupre SR, Druffel ERM. 2007. Ultra small-mass AMS ^{14}C sample preparation and analyses at KCCAMS/UCI Facility. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 259(1):293–302.

Sayle KL, Hamilton WD, Gestsdóttir H, Cook GT. 2016. Modelling Lake Mývatn's freshwater reservoir effect: Utilisation of the statistical program FRUITS to assist in the re-interpretation of radiocarbon dates from a cemetery at Hofstaðir, north-east Iceland. *Quaternary Geochronology* 36:1–11.

Schmitt J, Schneider R, Elsig J, Leuenberger D, Lourantou A, Chappellaz J, Kohler P, Joos F, Stocker TF, Leuenberger M, Fischer H. 2012. Carbon Isotope Constraints on the Deglacial CO2 Rise from Ice Cores. *Science* 336(6082):711–714.

Schoeninger M, DeNiro M, Tauber H. 1983. Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. *Science* 220(4604):1381–1383.

Schoeninger MJ, DeNiro MJ. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta* 48(4):625–639.

Schulting RJ, Ramsey CB, Bazaliiskii VI, Goriunova OI, Weber A. 2014. Freshwater reservoir offsets investigated through paired Human-Faunal ^{14}C dating and stable carbon and nitrogen isotope analysis at Lake Baikal, Siberia. *Radiocarbon* 56(3):991–1008.

Siani G, Paterne M, Arnold M, Bard E, Métivier B, Tisnerat N, Bassinot F. 2000. Radiocarbon reservoir ages in the Mediterranean Sea and Black Sea. *Radiocarbon* 42(2):271–280.

Siani G, Paterne M, Michel E, Sulpizio R, Sbrana A, Arnold M, Haddad G .2001. Mediterranean Sea Surface Radiocarbon Reservoir Age Changes Since the Last Glacial Maximum. *Science* 294(5548):1917–1920.

Soulet G. 2015. Methods and codes for reservoir–atmosphere ^{14}C age offset calculations. *Quaternary Geochronology* 29: 97–103.

Soulet G, Menot G, Bayon G, Rostek F, Ponzevera E, Toucanne S, Lericolais G, Bard E. 2013. Abrupt drainage cycles of the Fennoscandian Ice Sheet. *Proceedings of the National Academy of Sciences* 110(17):6682–6687.

Soulet G, Ménot G, Garreta V, Rostek F, Zaragosi S, Lericolais G, Bard E. 2011a. Black Sea "Lake" reservoir age evolution since the Last Glacial — Hydrologic and climatic implications. *Earth and Planetary Science Letters* 308(1–2):245–258.

Soulet G, Ménot G, Lericolais G, Bard E. 2011b. A revised calendar age for the last reconnection of the Black Sea to the global ocean. *Quaternary Science Reviews* 30(9–10):1019–1026.

Soulet G, Skinner LC, Beaupré SR, Galy V. 2016. A Note on reporting of reservoir ^{14}C disequilibria and age offsets. *Radiocarbon* 58(1):205–211.

Stuiver M, Polach HA. 1977. Discussion: Reporting of ^{14}C data. *Radiocarbon* 19(3):355–363.

van der Meer MTJ, Sangiorgi F, Baas M, Brinkhuis H, Sinninghe Damsté JS, Schouten S. 2008. Molecular isotopic and dinoflagellate evidence for Late Holocene freshening of the Black Sea. *Earth and Planetary Science Letters* 267(3–4):426–434.

Wood RE, Higham TFG, Buzilhova A, Suvorov A, Heinemeier J, Olsen J. 2013. Freshwater radiocarbon reservoir effects at the burial ground of Minino, Northwest Russia. *Radiocarbon* 55(1):163–177.

Yanko-Hombach V, Mudie PJ, Kadurin S, Larchenkov E. 2014. Holocene marine transgression in the Black Sea: New evidence from the northwestern Black Sea shelf. *Quaternary International* 345:100–118.

FIGURES AND FIGURE CAPTIONS

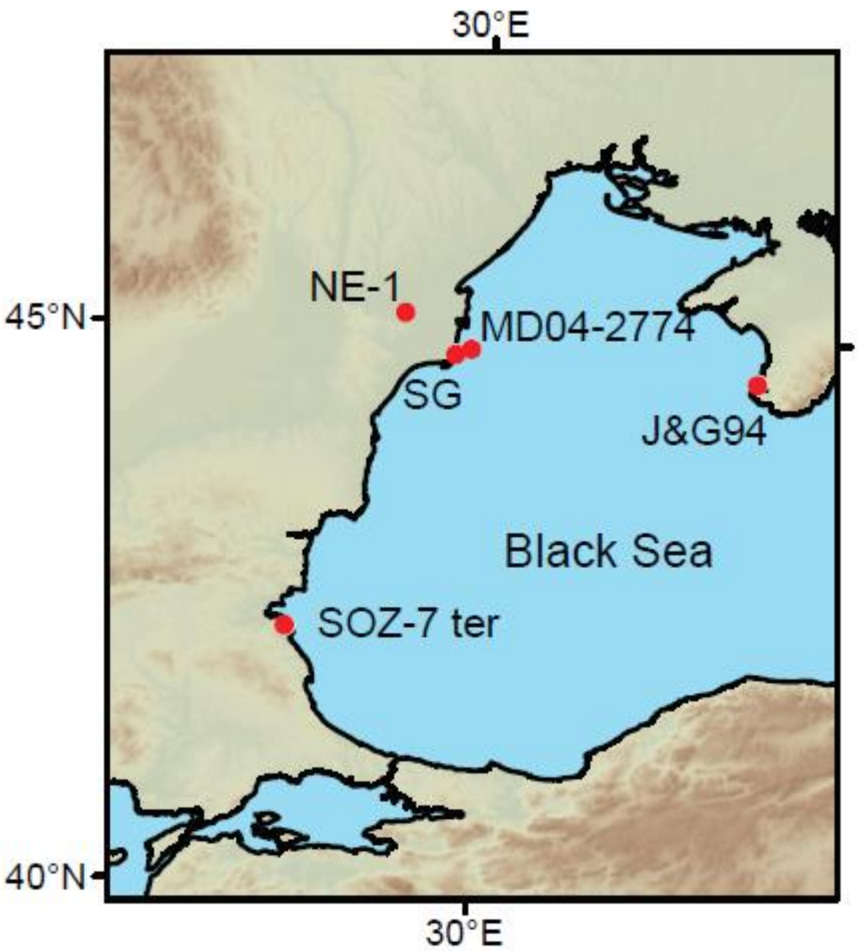

Figure 1: Western Black Sea area with sample locations and labels (Coordinates are available in Supplementary material; Table S1).

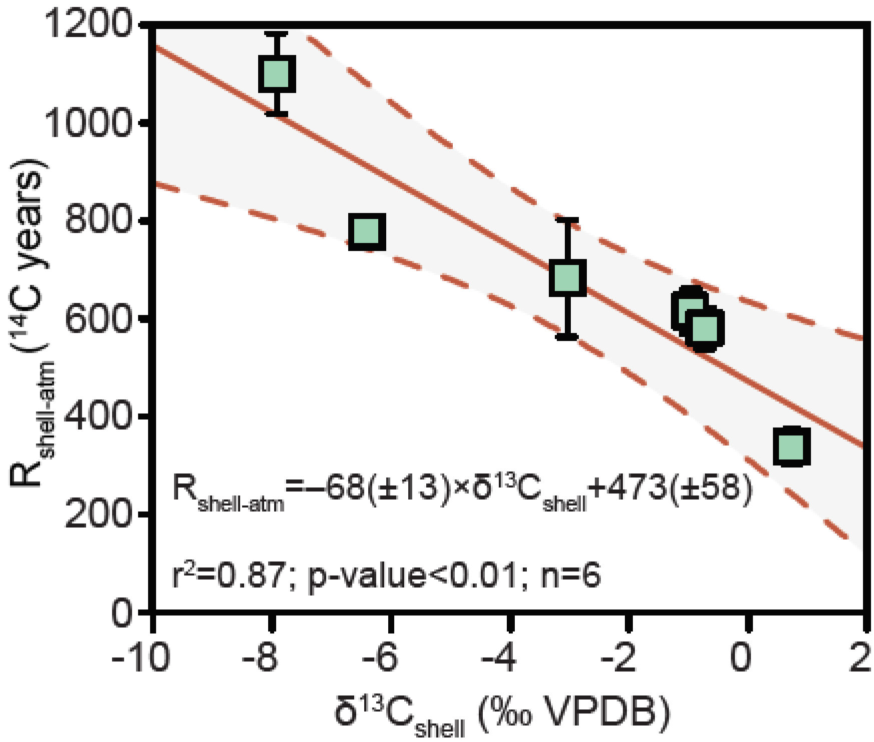

Figure 2: Linear regression of reservoir age offset ($R_{shell\text{-}atm}$) and $\delta^{13}C$ of Black Sea bivalve shells and its 95%-confidence interval.

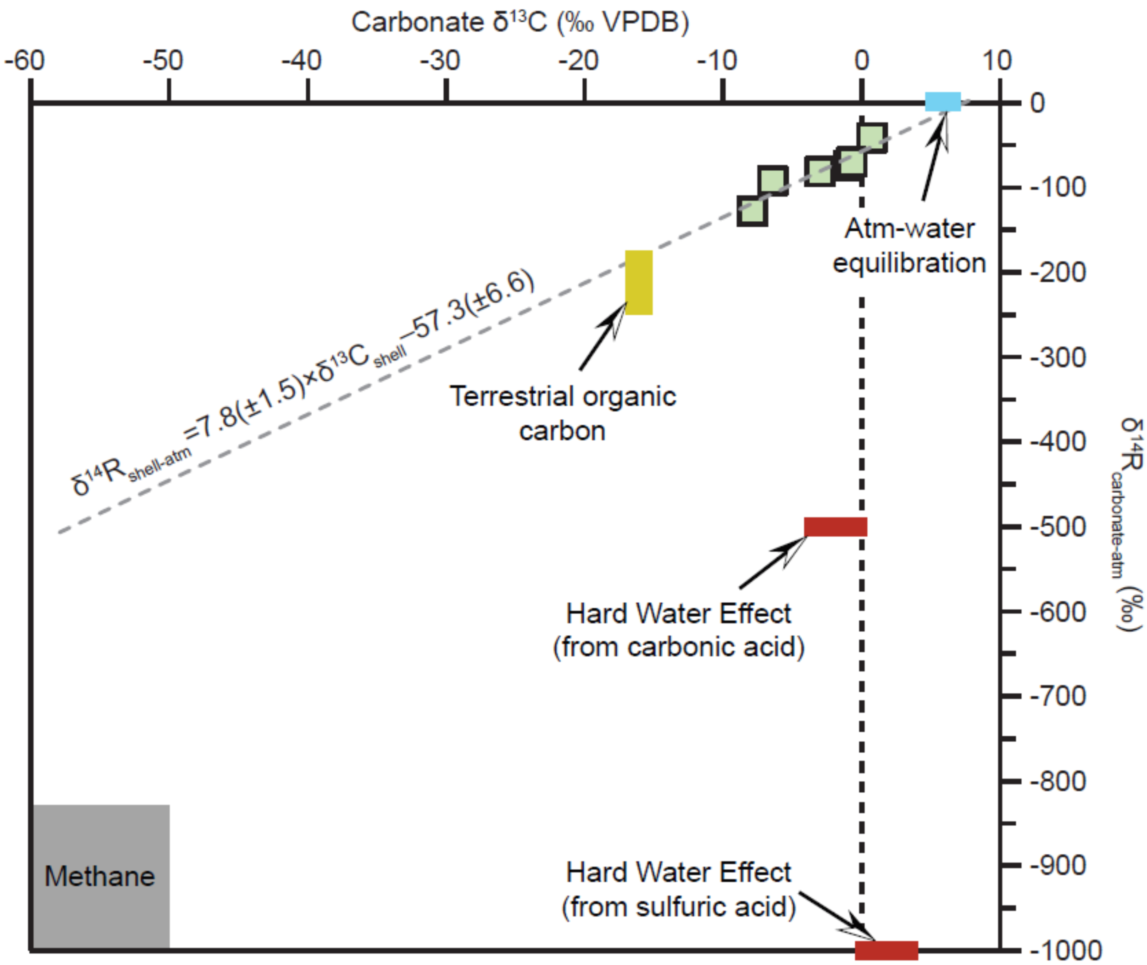

Figure 3: Linear regression of $\delta^{14}R_{shell\text{-}atm}$ and $\delta^{13}C$ of Black Sea bivalve shells (green squares; $r^2=0.87$; p-value<0.01; n=6) – same samples as in Fig. 2. Carbonate-equivalent endmembers of: i) CO_2 atmosphere-water equilibration (blue rectangle), ii) CO_2 from heterotrophic respiration of terrestrial organic matter supplied by rivers (yellow rectangle), iii) hard water effect from dissolution of outcropping carbonates by carbonic acid or sulfuric acid (red rectangles), and iv) CO_2 from oxidization of methane (grey rectangle). Vertical dotted line is $\delta^{13}C = 0$ ‰.

SUPPLEMENTARY MATERIAL – TABLE S1

Table S1. Coastal Black Sea ^{14}C data and reservoir age offsets

Area	Core/Name	Latitude	Longitude	Elevation (m)	Year[a] (AD)	Depth in core (cm)	Material	Lab #	Shell $\delta^{13}C$ (‰ VPDB)	Shell (ρ_{shell}) (^{14}C yr BP)	Material	Lab #	Atmospheric (ρ_{atm}) (^{14}C yr BP)	$R_{shell\text{-}atm}$ (^{14}C yrs)	$\delta^{14}R_{shell\text{-}atm}$ (‰)	Calibrated range (95%)[c] (cal yr BP)
Crimean coast	S&G94	44°40'00" N	33°30'00" E	0 to -5	1931	–	*Mytilus galloprovincialis*[b]	OS-718	0.72	490 ± 34	–	–	152 ± 7	338 ± 35	-41.2 ± 4.2	19[d]
Danube delta	NE-1	45°15'20" N	28°59'29" E	1	2005	350-351	*Dreissena polymorpha*	OS-117856	-6.44	4904 ± 19	Fragments of twigs	OS-117857	4126 ± 19	778 ± 27	-92.3 ± 3.0	[4537–4813]
Bulgarian coast	SOZ-7 ter(1)	42°21'55" N	27°42'30" E	1	2012	580-583	*Mytilus galloprovincialis*	Lyon-10874	-0.98	5138 ± 34	Charcoal	Lyon-10873	4524 ± 27	614 ± 43	-73.6 ± 5.0	[5053–5304]
Bulgarian coast	SOZ-7 ter(2)	42°21'55" N	27°42'30" E	1	2012	558-560	*Mytilus galloprovincialis*	Lyon-10872	-0.73	5056 ± 27	Charcoal	Lyon-10871	4475 ± 28	581 ± 39	-69.8 ± 4.6	[4978–5286]
Danube delta	MD04-2774	44°57'27" N	29°50'07" E	-30	2004	481-482	*Monodacna caspia*	OS-108017	-3.04	8940 ± 108	*Phragmites* reed	OS-107988	8258 ± 49	682 ± 119	-81.4 ± 13.7	[9040–9417]
Danube delta	SG	44°54'14" N	29°35'15" E	0.5	2007	4550-4551	*Dreissena sp.*	OS-77369	-7.92	10600 ± 60	Fragile foliar material from a peat layer	OS-65848	9500 ± 55	1100 ± 81	-128.0 ± 8.9	[10588–11081]

a Year of the collection of the material (the cores or the alive shell reported in Jones and Gagnon (1994)

b Pre-bomb shell collected alive in 1931 (Jones and Gagnon, 1994). The atmospheric age is inferred from Intcal13 (Reimer et al., 2013) using ResAge sofware (Soulet, 2015; Soulet et al., 2016).

c Calibrated using IntCal13 (Reimer et, 2013)

d Year AD 1931 is the year 19 calBP