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Fruits, fish and the introduction of pottery in the Eastern European plain: Lipid residue analysis of ceramic vessels from Zamostje 2

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ABSTRACT

The Neolithization of Northern Eurasia is marked by the emergence of pottery among hunter-gatherer societies. The driving forces behind the adoption of ceramic cooking vessels among non-agricultural societies remain unclear, although previous research, mainly in North East Asia (e.g. Japan, Korea and the Russian Far East), suggests that it was adopted as a specialist technology for processing aquatic resources, linked to the intensification of fishing activities and a move to sedentism. The stratified site of Zamostje 2 in the forest zone of the Volga-Oka region includes both aceramic Mesolithic and two early ceramic horizons dating to Early Neolithic (EN) and Middle Neolithic (MN). This provides a unique opportunity to look at the impacts of the adoption of pottery on the wider economy and determine whether pottery function changes over time. This was achieved through the analysis of lipids from 166 potsherds dating from the earliest phases (mid-6th millennium cal BC) to the MN (5th millennium cal BC). Contrary to our expectations, the pottery from the EN phase was used to process a broad range of foodstuffs including terrestrial resources, such as forest fruits, in addition to freshwater fish. In contrast, pottery from the MN phase was used exclusively for processing aquatic resources. The results show that in this case, pottery was adopted as a more general-purpose cooking container, at least in the earliest phases of use, and that a specialist function only emerged later.

1. Introduction: Hunter-gatherers pottery and Neolithization of Northern Eurasia

Archaeologists now acknowledge two contrasting processes of Neolithization. The classic definition of the Neolithic arose in Western Europe and involved the emergence of farming in the Near East, and the dispersal of a package of innovations including domestic crops and animals, village life and pottery into Northwest Europe. In contrast, archaeologists working in other parts of Eurasia define the onset of the Neolithic by the emergence of pottery cooking containers among hunter-gatherer societies, along with an increase in sedentism, emergence of new subsistence strategies with food storage and fishing intensification, and settlement at strategic locations giving access to a high biomass (Barnett and Hoopes, 1995; Kuzmin, 2006; Keally et al., 2007; Jordan and Zvelebil, 2009; Gibbs, 2015; Jordan et al., 2016). The earliest hunter-gatherer ceramic cooking vessels derive from East Asia. So far, the oldest pottery, securely dated, appears to have been made towards the end of the Late Pleistocene epoch, between 16,000 and 13,000 cal BC in South China, Japan, and the Amur River basin in the Russian Far East (Serizawa, 1979; Habu, 2004; Kudo, 2004; Kuzmin, 2006, 2017; Keally et al., 2007; Boaretto et al., 2009; Jordan and Zvelebil, 2009; Hommel, 2012). Although debated, it is suggested that pottery technology spread westward across Eurasia during the Holocene, eventually influencing several Northern European Mesolithic cultures (van Berg and Cauwe, 1998; Dolukhanov et al., 2005, 2009; Haaland, 2009; Jordan and Zvelebil, 2009; Gronenborn, 2011; Hartz

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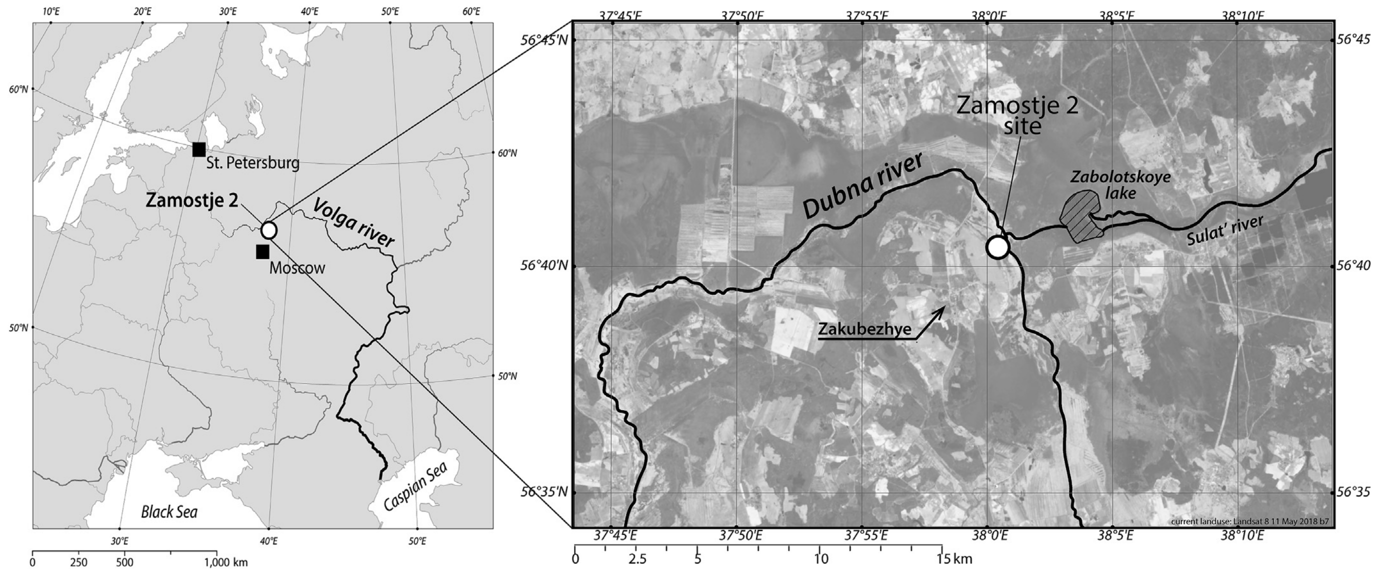

Fig. 1. Location of zamostje 2 along the Dubna river.

et al., 2012; Jordan et al., 2016). Its adoption may have had a major impact on prehistoric populations' lifeways as it became an essential everyday technology to cook and store foods. However, the motivations for the emergence, adoption and spread of pottery are still an ongoing debate in archaeology. The main questions are: (1) What was the function of the earliest ceramic vessels and did function change over time? (2) Was the adoption associated with substantial changes in economy, technology, society? (3) Did the adoption coincide with major environmental changes?

The increase of pottery abundance and its spread across large parts of Eurasia appears to occur in the Early Holocene, *ca.* 9700 to 5000 cal BC, and corresponds with a period of climate amelioration (Alley et al., 1993; Smith et al., 2011; Cummings, 2014). In Eurasia, numerous environmental changes occurred and rich new ecotopes emerged at this time. New vegetation (e.g. tundral, deciduous woodland) and fauna (e.g. reindeer, deer, wild boar, elk) spread to areas now free of ice (Khotinsky, 1993; Cummings, 2014; Zhilin, 2014) creating new opportunities for hunting and gathering. In the Postglacial landscapes, numerous chains of lakes were also formed and the rise of temperature and humidity that followed considerably enriched and diversified the productivity of the lacustrine ecosystems (Kulkova et al., 2001). In particular, the emergence of pottery appears to correlate with an increasing emphasis on exploitation of aquatic resources, combined with establishment of riverside and lake-edge settlements across large parts of Eastern and Western Siberian (Chairkina and Kosinskaia, 2009; Haaland, 2009; McKenzie, 2009), as well as Eastern Europe and the Baltic Sea Basin (Jordan and Zvelebil, 2009; Pesonen and Leskinen, 2009). These combined developments make it plausible to suggest that early pottery may have been linked to the processing of fish and other aquatic resources, and that ceramic cooking vessels may have offered certain advantages over other perishable container technologies.

Recent research has employed organic residue analysis of early pottery containers to clarify vessel function and explore some of the motivations that may have led to its emergence among hunter-fisher-gatherer societies (Jordan and Zvelebil, 2009). One clear pattern emerging from many of these regional studies is the apparent association between pottery and the processing of aquatic resources, including Northern and North-Eastern Europe (Craig et al., 2007; Isaksson, 2009; Oras et al., 2017), Sakhalin Island in the Russian Far East (Gibbs et al., 2017), Japan (Craig et al., 2013; Lucquin et al., 2016a, 2018), Korea (Shoda et al., 2017) and even in north-eastern North America (Taché and Craig, 2015). Interestingly, the close association between pottery and the processing of aquatic resources is observed even at sites where the faunal, botanical and artefactual evidence indicate exploitation of a much wider range of food resources (Lucquin et al., 2016a, 2018; Shoda et al., 2017; Jordan and Gibbs, 2018).

One area neglected by organic residue analysis is the vast forest zone of the Eastern European Plain. This extends from the Ukraine and western Belarus through European Russia to the Ural Mountains in the East. This region clearly participated in the wider uptake of pottery by local hunter-gatherers but the driving forces of its adoption are not yet properly understood. Our study analyses the function of pottery from a key Upper Volga site, Zamostje 2. This site is an ideal case-study due to its significant assemblage of well-preserved artefacts and ecofacts and an uninterrupted and well-dated stratigraphic sequence. Crucially, it captures the introduction of the first pottery culture in the Upper Volga Region, Central Russia, and its subsequent development during the MN (Lyalovo Culture). This gives us a rare opportunity to evaluate the economic impact of this technological change but also, using organic residue analysis, to reconstruct the use of early pottery and therefore to help understanding the motivations behind its adoption.

Our goals were twofold. Firstly, we aimed to determine whether the newly introduced pottery had a specific function, that is to say, was adopted for a specific reason. Secondly, we aimed to examine the evolution of pottery use over time from its emergence until the typological change concurrent with the MN. In this study we test the hypothesis that pottery was introduced within hunter-gatherer societies mainly for the processing of aquatic resources to then become a more general cooking container, as has been demonstrated elsewhere, e.g. Japan (Lucquin et al., 2016a).

2. The multi-layer waterlogged site of Zamostje 2, central Russia (Upper Volga Region)

Zamostje 2 is located *ca.* 110 km north of Moscow in the Sergiev Posad Region along the Dubna River (Fig. 1). The site was established on the edge of a vast lake basin with numerous river channels and was occupied during the Atlantic period from *ca.* 6600-4000 cal BC (Radu and Desse-Berset, 2013; Kulkova, 2014; Lozovski et al. 2014b). Two anthropogenic activity peaks are recorded, attributable to five successive cultural layers from the Late (Lower and Upper Layers from ca. 6500 to 5900 cal BC) and Final Mesolithic (from *ca.* 5900 to 5700 cal BC) to the Early (*ca.* 5700–5400 cal BC) and MN (*ca.* 5000-4000 cal BC) (Fig. 4) (Lozovski, 1996; Lozovski and Lozovskaya, 2013; Lozovski

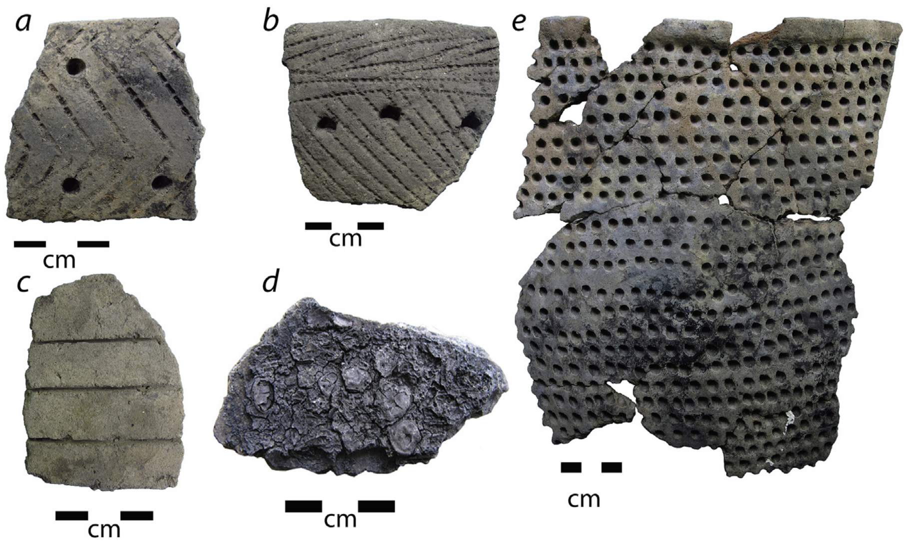

Fig. 2. Pottery from Zamostje 2: EN sherds with (a, b) long-comb decorations (late stage), (c) covered by incised lines (early stage); (d) Ceramic from EN layer (early stage) with remains of *Viburnum* (*Viburnum opulus* L.) berries in the crust, (e) MN Pottery with pit ornamentation.

Fig. 3. Plant and faunal remains and artefacts from Zamostje 2: (a) bone hooks from the EN, Final Mesolithic and mixed layers (from left to right), (b) bone net needles from the Upper Mesolithic, (c) floats from the Lower and Upper Mesolithic layer (from top to bottom), (d) tool made from beaver mandible from the Lower Mesolithic layer, (e) elk head figures made from elk antler from the Lower Mesolithic layer, (f) and (g) pine cone and nut remains from the Lower Mesolithic and mixed layer respectively, (h) barbed point from the EN.

et al., 2013a, 2013b; 2014b; Mazurkevich et al., 2013; Meadows et al., 2015).

Pottery constitutes the most numerous artefact in the Neolithic layers at Zamostje 2. In total, 18,300 sherds have been recovered from the EN layer, far more than similar sized excavations from contemporary sites in the Eastern European forest zone (Lozovski, 2003; Lozovski et al. 2014a; Mazurkevich et al., 2013; Mazurkevich and Dolbunova, 2015). In total, 26,911 sherds were recovered from the MN layer (Lozovski et al., 2015). The Upper Volga culture (UVC), attributed to the EN (Fig. 2; Fig. 4), was the first pottery culture in this region and consisted of several ceramic stages (Kostyleva 1986, 1987; 1994; 2003. Dolbunova et al. 2017). The ceramic material associated with the central zone of UVC culture, includes pottery that is either undecorated or decorated by rows of pointed impressions (Early Stage), "false-cord" decoration, incised lines, teethed-stamp impressions (end of Early Stage) (Kostyleva, 1994), short-teethed stamp impressions (Middle Stage) and finally different lengths of comb stamps (Late Stage) (Hartz et al., 2012; Mazurkevich et al., 2013; Lozovski et al. 2014a; Dolbunova et al., 2017). It is not clear whether these styles evolved within the region or developed from external influence(s) (Smirnov 2004; Mazurkevich et al., 2013).

Overall, the Zamostje 2 pottery assemblage is very fragmented, preventing an accurate quantification of the size and capacity of vessels through the different phases. The whole early Neolithic layer is "compressed" within a rather narrow horizon which complicated identification of specific functional/household areas or any specific "cultural" context, as well as identifying particularities of pottery type location. The EN phase comprises a wide range of pottery forms, mainly flat-bottomed cooking vessels and some bowls, most likely due to the extended period of time over which this material was deposited. Within the undecorated pottery, two completely different technological and morphological traditions were distinguished, which might indicate influences from different regions (Mazurkevich et al., 2013). Firstly, pottery which appeared here in the first half of the 6th millennium cal BC (Zaretskaya and Kostyleva, 2008) bears similarities with the pottery of the Middle Volga culture, Late Elshanian culture and Rakushechny Yar culture (Kostyleva, 2003). Later ceramic stage (Late Stage) might reflect influences from the Volga-Kama area.

The MN Phase is defined by the appearance of Lyalovo culture pottery vessels, characterised by pit and pit-comb ornaments across the whole of their exterior surfaces (Fig. 2e). This pottery style may appear to originate in the Volga–Oka interfluve (Lozovski et al., 2015; Vybornov et al., 2018). In contrast to the preceding UVC with a high proportion of flat-bottomed ware, Lyalovo complex vessels have roundish and conical bases (Lozovski et al., 2015; Vybornov et al., 2018). Nonetheless, vessels reconstruction, based on similar types of vessels found on other sites in this region, does not indicate a significant form change from early Neolithic to Lyalovo culture (Gurina and Krainov, 1996; Lozovski, 1996). On other Lyalovo culture sites some vessels were found fulfilled with fish scales (Gurina and Krainov, 1996).

Gathering practices were an important component of the Zamostje 2 inhabitants' lifeways. Up to 51 plant macrofossil taxa have been recovered and identified, including seeds and fruits (Fig. 3; Fig. 4). Most of the identified plant remains are from edible species or species that

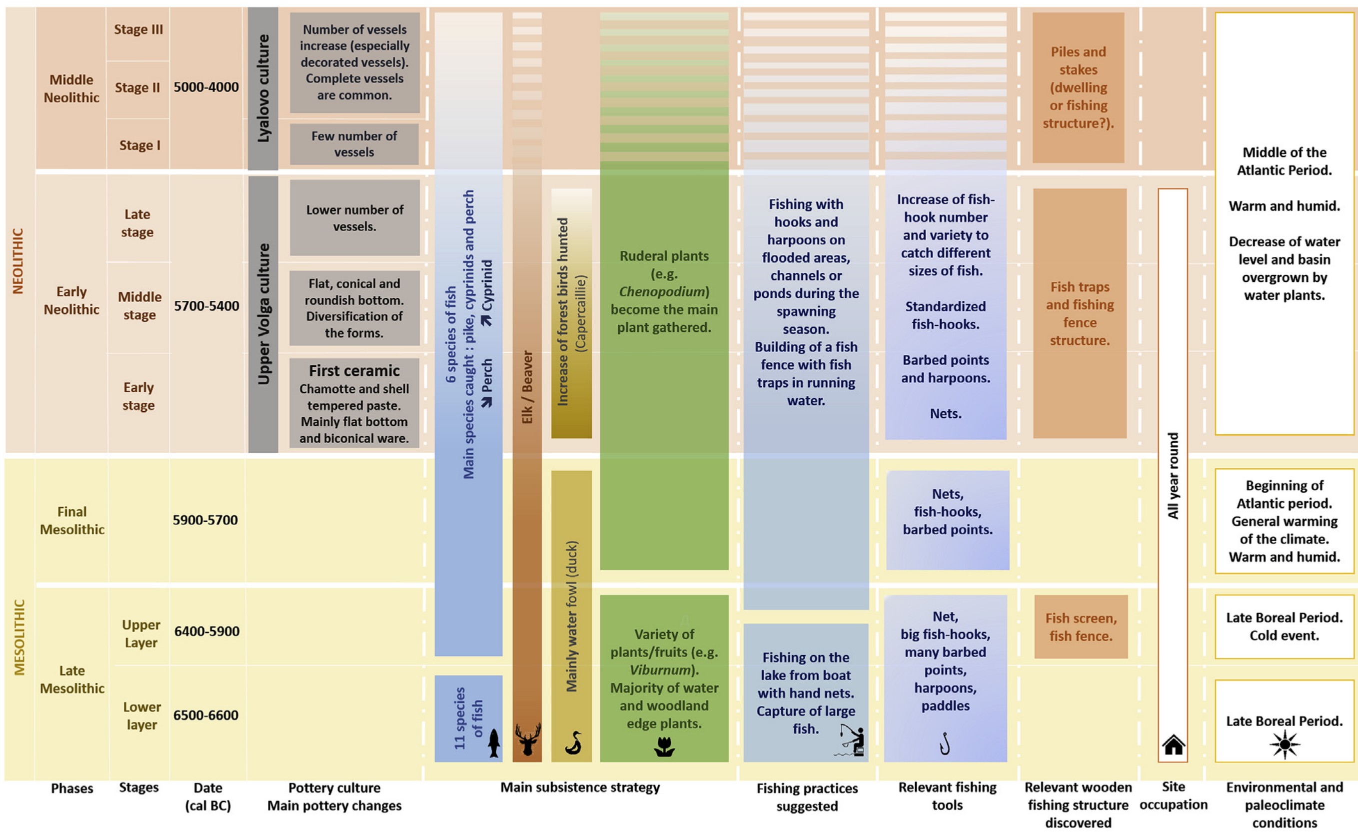

Fig. 4. Summary of archaeological evidence changes in Zamostje 2: dates, pottery cultures, subsistence strategies and environmental conditions. Based on the following references: Lozovski, 1996; Clemente et al., 2002; Ershova, 2013; Gyria et al., 2013; Lozovskaya and Lozovski, 2013, 2016; Lozovski et al., 2013a, 2013b; 2014a; 2014b; Mannermaa, 2013; Radu and Desse-Berset, 2013; Berihuete-Azorin and Lozovskaya, 2014; Leduc and Chaix, 2014, 2018; Mannermaa and Treuillot, 2014; Meadows et al., 2015; Berihuete, 2018; Ershova and Lozovskaya, 2018.

conceivably had a medicinal purpose (Berihuete-Azorin and Lozovskaya, 2014; Berihuete, 2018). Of the mammalian fauna, elk (*Alces alces*) and beaver (*Castor fiber*) were preferentially targeted, both easily accessible for hunting at the lake edge environment. Combined, these two species represent 70–90% of the mammal remains from Zamostje 2 (Leduc and Chaix, 2014, 2018). Birds such as waterfowl and forest species (mainly duck (*Anatidae*) and capercaillie (*Tetrao urogallus*) respectively) were exploited, with a rise of the latter in the Neolithic period, but this does not seem to have been a major economic activity (Mannermaa, 2013; Mannermaa and Treuillot, 2014). However, fishing at Zamostje 2 seems to have been a substantial subsistence activity, with hundreds of thousands of fish bones recovered (Lozovski et al., 2013a; Radu and Desse-Berset, 2013; Leduc and Chaix, 2014, 2018), as well as numerous fishing structures (e.g. fish traps, screens and fences) and fishing related tools (Fig. 3) (harpoons, barbed points, hooks, floats, small knots from nets, knives for fish processing) (Clemente et al., 2002; Lozovskaya and Lozovski, 2013; Lozovski et al., 2013b; Radu and Desse-Berset, 2013). From this evidence, two major fishing practices have been suggested. The first, during the earlier occupation of the site (i.e. Late Mesolithic), involved the use of hand nets from boats on the lake, whilst the second, from the Final Mesolithic, involved the inhabitants of Zamostje 2 fishing on flooded channels or ponds with hooks and harpoons and started to use stationary wooden structures (fish fences and traps) to conduct a more "passive fishing" (Gyria et al., 2013; Lozovski et al., 2013b) (Fig. 4). Apart from that, general food procurement, based on hunting, fishing and gathering, seems to have remained stable from the Late Mesolithic to Neolithic, despite the introduction of pottery.

3. Material and methods

3.1. Sampling strategy

A total of 240 samples were subjected to organic residue analysis, representing no fewer than 166 vessels. Of the pottery studied, 114 (63 from EN; 51 from MN) had charred residues indicating they had been used for cooking. A further 52 vessels (32 from EN; 20 from MN) without foodcrusts were selected. Pot sherds assigned from the Early to Late stage of EN (76 potsherds; 67 foodcrusts) and to the MN (45 potsherds; 52 foodcrusts) (Supplementary Material Table S1) were chosen in order to examine whether there was a temporal change in pottery use. In addition, lipids of modern plants (*Viburnum opulus* L.), ruminants (elk), wild non-ruminants (beaver) and fish (pike (*Esox lucius*), perch (*Perca fluviatilis*), cyprinids (Cyprinidae) and bream (*Abramis brama*)) from the region were also analysed for comparison with the archaeological samples (Supplementary Material Table S1).

3.2. Lipid residue extraction

Lipids were extracted and methylated from all samples following a modified one step acidified methanol protocol (Craig et al., 2013; Papakosta et al., 2015). A selection of samples (Supplementary Material Table S1) was subjected to solvent extraction following published methodologies (Charters et al., 1993; Regert et al., 1998; Stern et al., 2000; Gregg, 2009; Papakosta et al., 2015) in order to facilitate the detection of any triacylglycerols or wax esters. All extracts were analysed by Gas Chromatography-Mass Spectrometry (GC-MS) using different columns and modes to identify characteristic compounds (e.g. aquatic biomarkers). Where the lipid yield was sufficient, the methalonic acid extracts were subsequently analysed by Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS) in order to determine the carbon isotope values of the two most abundant fatty acids (C16:0 and C18:0). Foodcrusts were also analysed by Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS) to determine their stable nitrogen (δ15N) and carbon (δ13C) isotope values, as described previously (Craig et al., 2007; Lucquin et al., 2016a; Shoda et al., 2017). Further information is available in the Supplementary Materials.

4. Organic residue analysis of early and Middle Neolithic potsherds

4.1. Lipid quantification and characterisation

All the charred surface deposits (EN, $n = 67$; MN, $n = 52$) and absorbed residues (EN, $n = 76$; MN, $n = 45$) were extracted with the acidified methanol protocol and analysed by GC-MS and GC-C-IRMS to obtain specific compositional information. Additional solvent extraction ($n = 27$) was carried out where enough materials were present. Over 78% of the samples analysed provided interpretable lipid yields (potsherds > 5 μg/g; foodcrusts > 100 μg/g) (Evershed, 2008a; Lucquin et al., 2018), confirming the excellent preservation conditions at the Zamostje 2 site.

In general, the lipid profiles obtained (Fig. 5; Supplementary Material Table S1) contained saturated fatty acids, ranging from $C_{10:0}$ to $C_{30:0}$, mainly dominated by $C_{16:0}$. Monounsaturated fatty acid from $C_{14:1}$ to $C_{24:1}$ and branched fatty acids (C_{13}–C_{25}) were also identified. Dicarboxylic acids are present in more than 63% of the samples, mainly C_9 (azelaic acid), although a few samples ($n = 8$) contained a broader range (C_7–C_{22}). Cholesterol and its derivatives were broadly represented (n = 126 samples) in the samples, confirming that animal resources have been processed in the vessels.

A range of plant biomarkers such as diterpenes, mainly methyl dehydroabietate and 7-oxo-Dehydroabietate, which are markers of pine resin (Regert, 2004; Mitkidou et al., 2007; Jerković et al., 2011), terpenoids, plant sterols and their derivatives were identified (n = 140 samples) (Fig. 5). Interestingly, α-Amyrin, β-Amyrin, β-Amyrone terpenoids, common among angiosperm triterpenoids (Phillips et al., 2006; Courel, 2016), were identified. In some foodcrusts, these compounds were one of the most dominant peaks (Fig. 5), which in some cases may obtain up to 498 μg/g. These compounds are found in a wide range of plants and conceivably present in the sedimentary environment. However, their unusually high relative abundance (Fig. 5) shows that they are most likely endogenous and derived directly from prehistoric plant processing. Such biomarkers could be ascribed to the *Viburnum* berry, whose remains are macroscopically visible in some of the charred residues (Fig. 2d) (Berihuete-Azorín, 2016; Lozovski et al. 2014a). The analysis of modern *Viburnum* both undertaken here (Supplementary Material Table S1) and previously published (Powers and Powers, 1940) confirms that amyrin derivatives are present in *Viburnum*. There is a striking consistency between samples that contain the visible remains of *Viburnum* berries and amyrin derivatives. Indeed, 100% of samples containing *Viburnum* berries also contained terpenoid markers and the relative amount of amyrins in the total lipid extracts is significantly higher in the samples with visible *Viburnum* (mean = 3.6% of total lipid extracted) compared to those without visible *Viburnum* (mean = 1.9%; Mann-Whitney U = 82, z = 2.3, $p = 0.02$).

The presence of visible *Viburnum* has been reported at other UVC sites (Engovatova, 2000) suggesting it was widely exploited during this period. The fruits are rich in minerals and sugars and may have held medicinal properties due to the presence of antioxidants and its astringent and antispasmodic properties (Rop et al., 2010; Kalyoncu et al., 2013; Berihuete, 2018). Whilst they can be consumed raw, cooking removes their naturally sour taste (PFAF). Also, the fresh fruits can be used to obtain red dye (Berihuete, 2018, PFAF).

In addition, biomarkers for aquatic products were identified (Fig. 6, Supplementary Material Table S1) in many of the samples. The co-occurrence of ω-(o-alkylphenyl) alkanoic acids (APAAs) with 18 and 20 carbon atoms and isoprenoid fatty acids (phytanic, pristanic and 4,8,12-trimethyltridecanoic acid (TMTD)) are considered reliable indicators for aquatic processing in archaeological ceramics (Cramp and Evershed, 2014). Indeed, C_{20} APAAs result from thermal transformation of $C_{20:x}$ mono and polyunsaturated fatty acids, only present in appreciable concentrations in freshwater and marine animals (Evershed, 2008b; Baeten et al., 2013; Cramp and Evershed, 2014).

Whilst TMTD is mainly formed in aquatic resources, phytanic and pristanic acids are found in both aquatic and ruminant resources (Ackman and Hooper, 1968; Cramp and Evershed, 2014; Heron and Craig, 2015). To further distinguish the phytanic origin we examined the ratio of its diastereomers (3S,7R,11R,15-phytanic (SRR) and 3R,7R,11R,15-phytanic acid (RRR)) since the SRR-isomer is usually predominant (> 75.5% relative abundance) in aquatic animals (Lucquin et al., 2016b). Therefore, the detection of APAAs C_{18} and C_{20} along with either TMTD or a SRR% above 75.5% was used to confirm

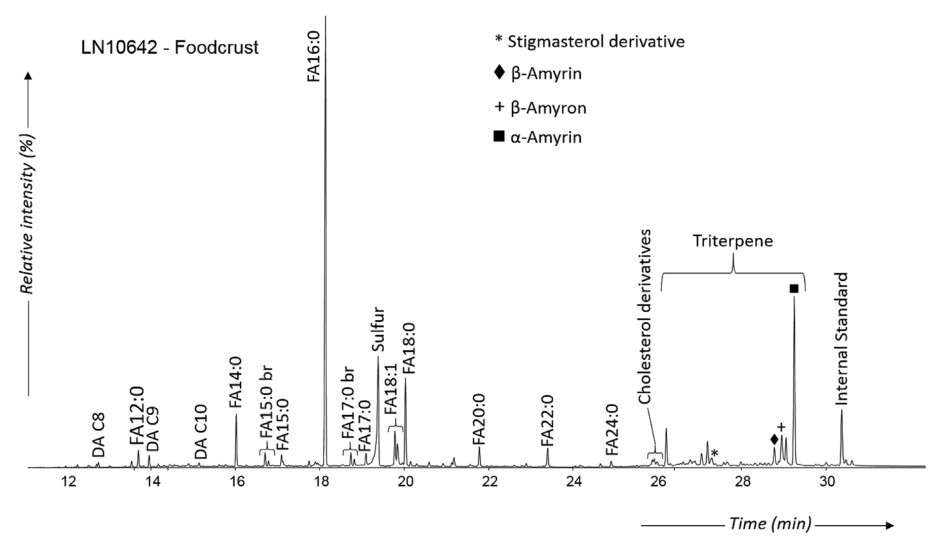

Fig. 5. Total ion current of an acid/methanol extract of the foodcrusts sample from pot LN10642, Zamostje 2. Partial gas chromatogram of lipid showing saturated fatty acids (FA), diacids (DC), branched chain fatty acids (br), long-chain unsaturated fatty acids, sterols and triterpenes whose amyrin derivatives are indicated.

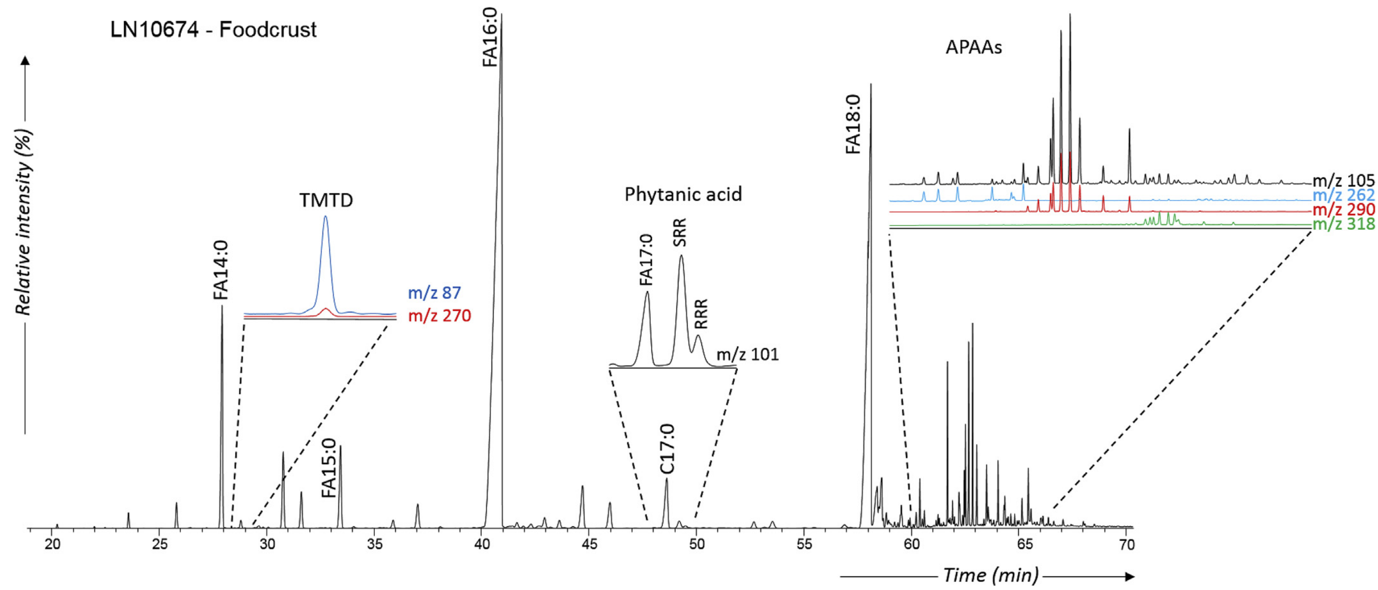

Fig. 6. SIM chromatogram indicating the aquatic biomarkers from sample LN10642 analysed on the DB-23 column. The ω-(o-alkylphenyl) alkanoic acids were identified with ions m/z 105, 262, 290, 318, with the last three ions corresponding to the carbon length C_{16}, C_{18}, and C_{20} respectively. 4,8,12- trimethyltridecanoic acid was monitered with ions m/z 87, 270 and phytanic acid with ions m/z 101, 171, 326. The m/z 101 ion chromatogram shows the diastereomers of phytanic acid (SRR and RRR), which allowed us to calculate the relative abundance these diastereomers.

the presence of aquatic products in pots. In total, 68% of the samples analysed satisfied the full molecular criteria for the processing of aquatic products in archaeological pottery.

Interestingly, the results indicate an increase in the processing of aquatic resources during the MN. In total, 81% of these samples contained the full set of aquatic biomarkers, compared to only 55% of the EN samples (Table 1). An increase in the proportion of samples containing aquatic biomarkers appears to begin at the end of the EN (Table 1). The occurrence of plant biomarkers follows an opposite trend, with a higher proportion of EN vessels yielding these compounds compared to the Lyalovo vessels (Table 1). The main plant biomarkers, amyrin derivatives, decline gradually through the EN and are absent in the MN pottery.

4.2. Stable carbon isotope analysis of individual fatty acids

To provide more information about the origin of the lipid residues, stable carbon isotope analysis of palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids was undertaken using GC-C-IRMS. Analyses were carried out on all samples yielding sufficient quantities of fatty acids; 170 samples in total, which included 100 EN and 70 MN samples. In Fig. 7, the $\delta^{13}C$ values of the $C_{16:0}$ acid are plotted against the difference between the carbon isotope values of two main fatty acid ($\Delta^{13}C = C_{18:0}$-$C_{16:0}$). This approach enables us to distinguish ruminant adipose and dairy fats from other non-ruminant sources (Dudd, 1999; Craig et al., 2012, 2013; Cramp et al., 2014; Colonese et al., 2015; Taché and Craig, 2015; Lucquin et al., 2016a). We also include some modern samples from the Zamostje 2 area (Supplementary Material Table S1). In the majority of cases the observed values are consistent with a non-ruminant source. The large carbon isotope range and negative correlation between $\delta^{13}C_{16:0}$ and $\Delta^{13}C$ is however perplexing, and points to a mixture of different sources, which could include freshwater fish with different isotope values, plants or non-ruminant terrestrial animals, such as beaver, which are abundant in the faunal assemblage. Analysis of the collagen extracted from the bones of pike (*Esox lucius*; $n = 10$) and cyprinids (Cyprinidae, $n = 10$), shows a wide range of $\delta^{13}C$ values (pike between −23.3 and −19.5‰; cyprinids between −27.2 and −22.3‰) (Meadows et al., 2019 *In prep*). The more enriched $\delta^{13}C_{16:0}$ values could have been obtained by the processing of ruminants consuming C_4 plants (Gregg, 2009; Craig et al., 2012). However, the absence of C_4 plants in this region means that ruminant products do not adequately explain the variation; two modern local elk samples are plotted to illustrate this point. There is also no difference in the fatty acid carbon isotope value by period or by presence/absence of aquatic-derived lipids. However, the $\delta^{13}C_{16:0}$ and $\delta^{13}C_{18:0}$ values of EN samples show a greater variability compared to the MN (Mann-Whitney test $U = 2846$, $z = 2.07$, $p = 0.04$; $U = 2780$, $z = 2.28$, $p = 0.02$ respectively), which presumably reflects the wider range of foodstuffs processed in these vessels.

Table 1
Table summarizing the proportion of plant and aquatic biomarkers detected in the samples according to the period and comparing the proportion of the main plant biomarkers (amyrin and derivatives) and aquatic biomarkers through the different EN stages and MN.

Period	Stage	Samples analysed	Yielding lipids (%)*	Yielding triterpenes (%)	Yielding amyrin and derivatives (%)	Yielding diterpenes (%)	Yielding plant sterols (%)	Yielding aquatic biomarkers (%)
Early Neolithic	Early stage	52			52			53
	Middle stage	44			47			49
	Late stage	10			30			73
Total		**143**	**82**	**65**	**45**	**32**	**22**	**55**
Middle Neolithic		97	86	23	0	23	12	81

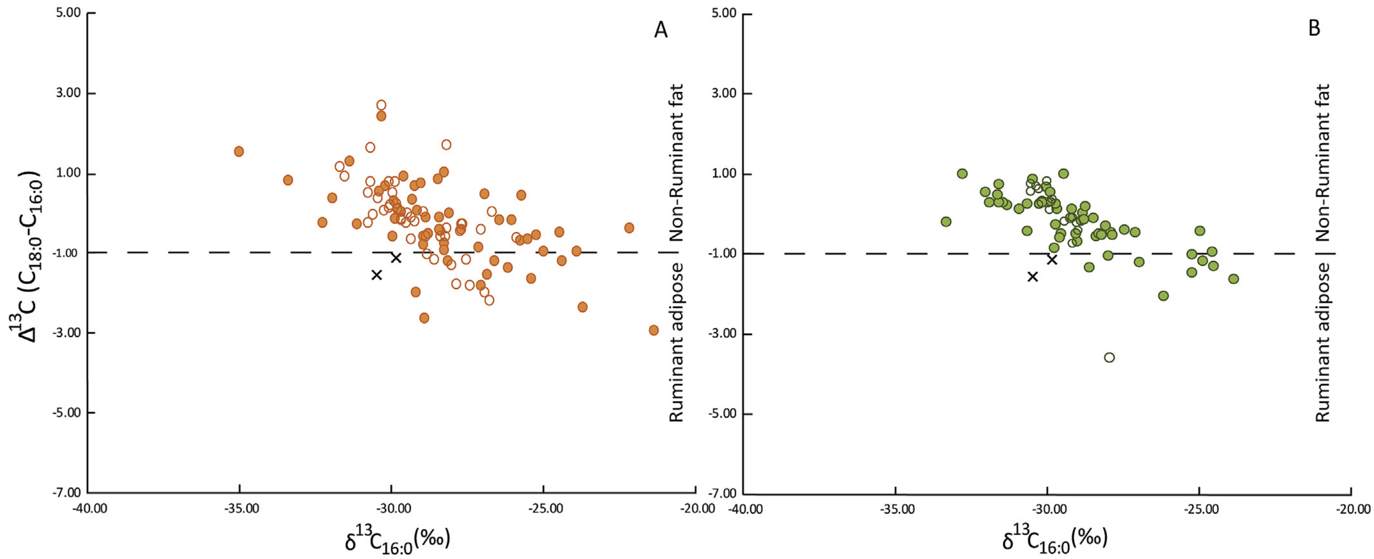

Fig. 7. $\Delta^{13}C$ ($\delta^{13}C_{18:0} - \delta^{13}C_{16:0}$) values against $\delta^{13}C_{16:0}$ values obtained from foodcrusts and sherds from (A) EN (n = 100) and (B) MN (n = 70) samples. Crosses indicate modern elk from the Zamostje 2 region. Samples with the full and partial set of aquatic biomarkers are shown by filled circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.3. Bulk isotope analysis of charred surface deposit

Bulk stable isotope values and the elemental analysis for the charred surface deposits (n = 108) are plotted in Fig. 8 and reported in Table S1 (Supplementary Material). The bulk $\delta^{13}C$ isotope values from Zamostje 2 range from −23.3 to −29.6‰ and are indicative of a range of terrestrial C_3 plants, terrestrial mammals and freshwater fish, but these data are also influenced by the relative degree of preservation of lipids, carbohydrates and proteins making them difficult to interpret (Craig et al., 2007, 2011; 2013; Yoshida et al., 2013; Heron et al., 2016). Conversely, the nitrogen present in the charred products is derived from proteins and therefore the $\delta^{15}N$ values reflect the trophic level of the organisms processed in the vessels. Thus, high $\delta^{15}N$ values, above *ca.* +7.0–9.0‰, are usually characteristic of aquatic resources (Dufour et al., 1999; Craig et al., 2013; Choy et al., 2016), whereas lower ones are consistent with terrestrial organisms (Yoneda et al., 2004; Craig et al., 2007; Yoshida et al., 2013). The atomic C:N ratio is indicative of the amount of protein versus other macromolecules (carbohydrates and lipids). Generally animal tissues, enriched in protein, will have lower C:N ratios compared to plant tissues, enriched in carbohydrates such as starch and cellulose (Yoneda et al., 2004; Yoshida et al., 2013; Choy et al., 2016; Heron et al., 2016; Lucquin et al., 2016a).

Interestingly, there is a clear correlation between the bulk $\delta^{15}N$, C:N ratio (Table 2) and molecular characterization of these samples. Pottery vessels with high C:N ratios tend to have lower $\delta^{15}N$ values, indicating that they are mainly derived from plant products, supported by a greater proportion containing amyrins (Fig. 8a). Samples with lower C:N ratios and higher $\delta^{15}N$ values have a greater proportion of aquatic-derived lipids (Fig. 8b), suggesting they were used for processing fish. Furthermore, there is a clear difference in $\delta^{15}N$ and C:N ratios between EN and MN pottery. The former has more variable values, indicating that a wider range of foodstuffs were processed, which must include mixtures of aquatic and plant products (and possibly terrestrial animals). The later pots have a greater proportion of aquatic products, consistent with increasing specialisation focused on fish processing. These data contrast with the lipid-specific carbon isotope measurements made on the same samples which show no clear difference between periods (Fig. 7). The reason for this is that the bulk isotope measurements reflect greater variation in the contribution of other macronutrients (carbohydrates and proteins) to the potsherds compared to the lipid-specific measurements.

5. Discussion

The main aim of our study was to examine the function of pottery recovered from Zamostje 2 following its introduction, during the early Neolithic period (*ca.* 5700-5400 cal BC), and its subsequent development in the MN (Lyalovo period, after 5000 cal BC). Based on expectation from previous studies of pottery use by Eurasian hunter-gatherers (Craig et al., 2007, 2013; Taché and Craig, 2015; Lucquin et al., 2016a; Gibbs et al., 2017; Oras et al., 2017; Shoda et al., 2017), we hypothesized that the first pottery in this region would have been used for processing aquatic resources. The organic residue analyses we undertook refute these assumptions. All the evidence shows that pottery at Zamostje 2 initially was used to process a wide range of foodstuffs, certainly including aquatic and terrestrial plants products and possibly also terrestrial animals. It is only in the MN that a different pattern emerges, with almost all the samples analysed showing a clear aquatic molecular and isotopic signature, pointing to specialisation in the use of pottery at this time.

The new evidence we have generated from the use of pottery contrasts with faunal analyses so far undertaken on the Zamostje 2 assemblage. These data show that fishing was a significant economic activity even before the introduction of pottery, during the Mesolithic period (*ca.* 6500-5700 cal BC), and that the importance of this activity did not fundamentally change throughout the EN period. Research on the MN faunal assemblage is not complete, but the quantity of fish bone compared to terrestrial species remains similarly high (Radu and Desse-Berset, 2013). Locally, the paleoenvironmental records show dense forest cover during EN with greater afforestation of the lake shores, and a change to a more marshy landscape during the MN (Ershova and Lozovskaya, 2018). More broadly, the introduction of pottery is not associated with significant change in climate and occurs several centuries after the warming events marking the Holocene Thermal Maximum, *ca.* 6000–2000 cal BC (Heikkilä and Seppä, 2010). In summary, resources available in the Neolithic inhabitants of Zamostje 2 were readily available to the Mesolithic hunter-gatherers that preceded them. How then do we explain the appearance of pottery at Zamostje 2 and its changing use in the MN?

One hypothesis is that the pottery use reflects a change in culinary practices rather than any major shift in the economic strategy. Indeed, there is evidence that fish were prepared and consumed without being cooked or extensively processed during the aceramic Mesolithic phases.

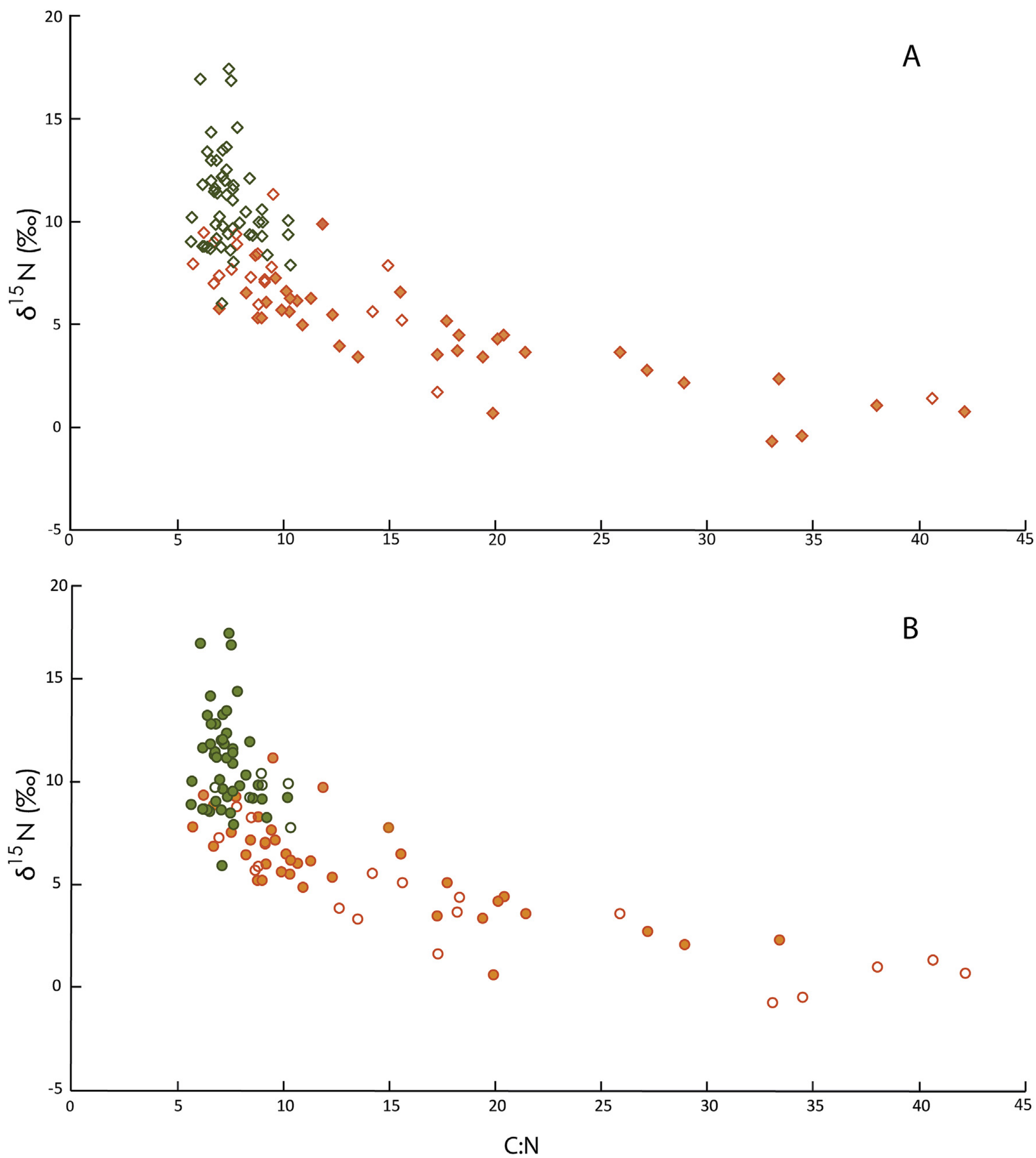

Fig. 8. Plot of $\delta^{15}N$ bulk isotope values against C:N ratio obtained from foodcrusts samples from Early ($n = 56$) in orange and MN ($n = 52$) in green showing (A) samples in which amyrin derivatives were detected by filled diamonds, (B) samples with the full set of aquatic biomarkers by filled circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Human and dog coprolites at the site frequently contained fish bone, suggesting that fish was eaten whole (Engovatova and Hrustalyov, 1996; Lozovski et al., 2013a; Radu and Desse-Berset, 2013). The eggs of parasitic worms (*Diphylobothrium latum*) have also been interpreted to suggest that fish had not been subjected to prolonged heat treatment (Engovatova and Hrustalyov, 1996; Lozovski et al., 2013a). In this scenario, the routine practice of cooking fish in pottery only occurred in the Middle Neolithic, but certainly fish were regularly consumed in other ways well before.

If one accepts this scenario, two interpretations of this shift

Table 2
Mann-Whitney *U* test showing correlation between the bulk $\delta^{15}N$, C:N ratio and molecular characterization. The test revealed a significant difference of $\delta^{15}N$ and C:N ratio between EN and MN samples, samples with or without the full set of aquatic biomarkers and samples with or without amyrin derivatives. However, there is no correlation between $\delta^{13}C$ values and periods or molecular characterization of samples.

Category	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	C:N ratio
Period (EN vs. MN)	U = 118; z = 8.2; $p < 0.01$	U = 1217; z = 1.3; $p = 0.2$	U = 339; z = 6.8; $p < 0.01$
Aquatic biomarkers (presence vs. absence)	U = 457; z = 3.9; $p < 0.01$	U = 949; z = 0.1; $p = 0.9$	U = 397; z = 4.3; $p < 0.01$
Amryin derivatives (presence vs. absence)	U = 156; z = 7.4; $p < 0.01$	U = 1126; z = 1.0; $p = 0.3$	U = 198; z = 7.1; $p < 0.01$

occurring in the MN can be proposed. It could be, firstly, related to a “simple” change in culinary practices brought by the new population, which also introduced a new pottery tradition. On the other hand, it can reflect new economic strategies, such as changes in the scale of exploitation or changes in the seasonal occupation of the site. Analysis of the fish bones, recovered from the EN and MN layers, shows that fishing mainly occurred during the spring and summer (Lozovski et al., 2013a; Radu and Desse-Berset, 2013). As the residue data we have generated show that new type of pottery containers found from the end of 6th millennia-5th millennia (later EN/Lyalovo culture) were used almost exclusively for fish processing, we suggest that the site became a more specialised seasonal fishing station at this point. Indeed, processing fish in pottery to make storable products (e.g. fermenting or rendering to make oils) may have been needed to deal with the seasonal surplus, although further analysis of terrestrial fauna and artefactual remains from the MN layers, which are lacking at the moment, is needed to confirm or refute this hypothesis. In contrast, during the Mesolithic and EN, Zamostje 2 appears to have been occupied all year round (Lozovski et al., 2013a), which would be more in keeping with a broader range of products identified in the EN pottery, such as *Viburnum* fruits that ripen in autumn (Berihuete, 2018).

Finally, the lack of a major shift in subsistence strategies associated with the introduction of pottery suggests it was incorporated into existing cultural and economic practices rather than having a major transformative effect. Pottery may have simply fulfilled a range of functional niches previously occupied by perishable containers, such as baskets, pits or other organic containers, such as those made from wood, tree bark or animal tissue. Ethnographic evidence shows that foodstuffs can be easily heated in such artefacts, negating a specific need for pottery (Driver and Massey, 1957; Leroi-Gourhan, 1973). This ‘software’ to ‘hardware’ transition, although conspicuous by its visibility in the archaeological record, may have therefore had far less actual impact on hunter-gatherer lives than supposed, perhaps only resulting in marginal gains in terms of cooking performance and durability that out-weighed the production costs.

It is interesting to note, however, that at Zamostje 2, where the conditions are highly conducive to the preservation of wood and bark at least, relatively few containers have been found in the Mesolithic layers (Lozovski, 1996; Lozovski and Ramseyer, 1998; Lozovskaya and Lozovski, 2016), and none are directly analogous in form to the ceramic vessels that emerge. Similarly, container finds from the Mesolithic wetlands sites excavated across the region are extremely rare (Burov, 1989; Koltsov, 1989; Oshibkina, 2006). While other forms of container that might not have survived can be proposed, alternative explanations evoking non-functional attributes of pottery (e.g. Hayden, 1998) are perhaps needed to explain its adoption in some regions of Eastern Europe (Mazurkevich and Dolbunova, 2015).

6. Conclusions

The new data generated from analysis of the Zamostje 2 pottery assemblage do not support our hypothesis that the introduction of pottery in Holocene Eurasian hunter-gatherer societies was driven by a specialist need to process aquatic resources. The zooarchaeological and artefactual data show that fishing was already well established before the arrival of pottery and remained important well after the onset of Holocene Thermal Maximum climate optimum. Interestingly, the first pottery of the Early Neolithic was used to process a variety of foodstuffs, including fruit and other terrestrial resources. A shift towards specialisation in the ceramic use, focused on fish processing only appears ca 700 years later, in the Middle Neolithic. Therefore, at Zamostje there appears to be very little in the way of revolutionary change associated with the first pottery and the onset of the Neolithization. It is also striking that the new technology was adopted with minimal change in other aspects of the economy and society. It appears that pottery was simply adopted as a multi-functional cooking container. Surprisingly, there is little evidence of perishable containers in the Mesolithic deposits at Zamostje, despite the excellent potential for preservation, suggesting that pottery did not simply replace non-ceramic analogues. The use of pottery for processing and combining foods through sustained heating may instead have been linked to social motivations, e.g. preparing dishes for elaborate feasting (Hayden, 1998), or aesthetic reasons that are not directly related to a wider economic shift, as has been suggested for other prehistoric foragers (Saul et al., 2013). Clearly, further work will be needed across the Eastern European Plain to confirm whether the patterns of early pottery use noted at Zamostje are exceptional or perhaps part of a wider trend which involves pottery being adopted as a new way of cooking and combining a diverse array of foodstuffs.

Statistical

Statistical tests were performed using *Past* (version 3.21).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quaint.2019.05.008.

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