Exploring the emergence of an 'Aquatic' Neolithic in the Russian Far East: organic residue analysis of early hunter-gatherer pottery from Sakhalin Island

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The Neolithic in north-east Asia is defined by the presence of ceramic containers, rather than agriculture, among hunter-gatherer communities. The role of pottery in such groups has, however, hitherto been unclear. This article presents the results of organic residue analysis of Neolithic pottery from Sakhalin Island in the Russian Far East. Results indicate that early pottery on Sakhalin was used for the processing of aquatic species, and that its adoption formed part of a wider Neolithic transition involving the reorientation of local lifeways towards the exploitation of marine resources.

Keywords: Sakhalin Island, Neolithic, organic residue, hunter-gatherers, aquatic resources

Sample preparation and Analysis

This study includes 42 samples of lipid extracts taken from 41 pottery sherds representing three Neolithic sites from Sakhalin Island. Samples include both surface food crusts (n=15) and ceramic matrix (powder) that was drilled from the interior surfaces of sherds (n=27). The food crusts were sent to the University of York for EA-IRMS and GC-MS. The drilled (absorbed) samples were sent to Stockholm University for GC-MS analysis. Subsequent GC-c-IRMS analysis of certain absorbed residues (n=11) was performed at the University of Liverpool. For one sherd from Slavnaya 4 we sampled foodcrusts from both the interior and exterior surfaces (samples 101 and 101e). All of the other food crusts were derived from interior surfaces.

EA-IRMS of charred surface deposits

Foodcrusts (~2 mg) were crushed and homogenised using an agate pestle and mortar. These were directly analysed by elemental analysis - isotope ratio mass spectrometry (EA-IRMS) to determine their bulk stable carbon ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) composition, as previously reported (Craig *et al*. 2007). Samples yielding less than 1% N were not used and instrument precision on repeated measurements was ±0.2‰ (s.e.m.). $\delta^{13}C$, $\delta^{15}N$ = [(Rsample/Rstandard−1)] × 1,000, where R = $^{13}C/^{12}C$ and $^{15}N/^{14}N$. All sample measurements are expressed in per mil relative to the standard for $\delta^{13}C$ is Vienna PeeDee Belemnite (V-PDB) and the standard for $\delta^{15}N$ is air N_2, respectively.

Lipid extraction of ceramic powders

The ceramic powders (~0.5 g) were extracted with 1.5ml of a mixture of chloroform and methanol (2:1, v:v) using sonication (2x15min). The samples were then centrifuged (3000rpm, 30min). The clear extracts were transferred to vials and the solvent evaporated under a gentle stream of nitrogen. The lipid residues were treated with bis(trimethylsilyl)trifluoracetamide containing 10% (v) chlorotrimethylsilane at 70°C for 20min to produce trimethylsilyl (TMS) derivatives, which were then dried under nitrogen. The derivatised extracts were re-dissolved in n-hexane and analysed by GC-MS.

From these a selection of ceramic samples (87, 96, 103, 108, 114, 119) with good lipid yields was hydrolyzed in 1ml 0.5M NaOH in methanol at 70°C for 1h to release bound fatty acids. When the mixture had cooled it was neutralized using 6M HCl. The free fatty acids was extracted using n-hexane three times, the extracts recombined and dried under nitrogen. The dry free fatty acids were treated with boron triflouride (25%) in methanol at 70 °C in order to produce methyl esters. The fatty acid methyl esters were extracted using n-hexane and dried

under nitrogen. The derivatised extracts were re-dissolved in n-hexane and analysed by GC-MS and GC-c-IRMS using the procedure described below.

Lipid extraction of foodcrusts and some ceramic powders

The foodcrusts and several ceramic powders yielding no lipid residues from the solvent extraction were extracted using a one-step acid catalyzed extraction and derivatization technique (Papakosta *et al*. 2015). Foodcrusts (5mg) or the ceramic powder was homogenized with CH_3OH (1ml) in an ultrasonic bath and concentrated H_2SO_4 (200µl) was added as catalyst. After heating for 4h at 70°C, n-hexane was added, shaken vigorously, and separated from the methanol phase after centrifugation (5min) to obtain the methylated fatty acids. The procedure was repeated twice, and the combined extracts were dried. The derivatised extracts were re-dissolved in n-hexane and analysed by GC-MS and then analysed by GC-c-IRMS using the procedure described below. Alternatively, lipids from homogenized charred deposits were extracted by alkali saponification [2mL of sodium hydroxide (5% [wt/vol] in methanol) for 2h at 70°C]. Saponified extracts were cooled, neutral lipids were removed (n-hexane, 3 × 2mL), the extracts were acidified with HCl and the acid fraction was extracted (n-hexane, 3 × 2mL) and methylated using BF3-methanol complex [14% (wt/vol), 200µL, 1h, 70°C].

GC-MS analysis of ceramic powders

Molecular analyses were initially performed on a HP 6890 Gas Chromatograph fitted with a SGE BPX5 fused-silica capillary column (15m× 220µm×0.25µm), coupled to a HP 5973Mass Selective quadrupole detector. All derivatised extracts were injected through a Merlin Microseal High Pressure Septum in pulsed splitless mode (pulse pressure 17.6Psi, 325°C). The column oven was temperature programmed starting with an initial isothermal at 50°C for 2min. Then, the temperature increased with 10°C per minute to reach 360°C, and remained at this temperature for 15min. Helium was used as carrier gas, with a controlled constant flow of 2.0ml/min. The ion source was maintained at a temperature of 230°C, ionization and fragmentation were accomplished by electron impact (70eV). The mass filter was set to scan between m/z 50 and 700, with a scan rate of 2.29 scans per second.

All solvents used were of Pro Analysis grade. All glassware was rinsed with concentrated nitric acid prior to use and experimental blanks were run in parallel with the samples.

GC-MS analysis of foodcrusts

GC-MS was carried out on all samples using an Agilent 7890A Series chromatograph attached to an Agilent 5975 C Inert XL mass-selective detector with a quadrupole mass analyser (Agilent technologies, Cheadle, Cheshire, UK). A splitless injector was used and

maintained at 300°C. The carrier gas used was helium, and inlet/column head-pressure was constant. The GC column was inserted directly into the ion source of the mass spectrometer. The ionisation energy of the mass spectrometer was 70eV and spectra were obtained by scanning between m/z 50 and 800.

Screening was performed using a DB-23, (50%-Cyanopropyl)-methylpolysiloxane column (60m x 0.25mm x 0.25µm; J&W Scientific, Folsom, CA, USA). The temperature for this column was set at 50°C for 2 minutes, then raised by 10°C min^{-1} until 100°C, then raised at 4°C min^{-1} until reaching 250°C where it was held for 20 minutes. The carrier gas used was helium with a flow rate of 2.3mL min^{-1}.

GC-c-IRMS analysis

Carbon stable isotope ratios were determined on two fatty acid methyl esters, methyl palmitate ($C_{16:0}$) and methyl stearate ($C_{18:0}$), in each extract using a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) linked to a Trace Ultra gas chromatograph (Thermo Fisher) with a ConFlo IV interface (Cu/Ni combustion reactor held at 1000°C; Thermo Fisher). All samples were diluted with hexane and subsequently 1µL of each sample was injected into a DB5 fused-silica column. The temperature was set for 1 min at 45°C, and raised by 6°C min^{-1} to 295°C, where it was held for 15 min. The carrier gas was ultra-high-purity grade helium at a flow rate of 1.4mL min^{-1}. The eluted products were combusted to CO_2 and ionised in the source of the mass spectrometer by electron ionisation. The ion intensities of *m/z* 44, 45, and 46 were monitored in order to automatically compute the $^{13}C/^{12}C$ ratio of each peak in the extracts. Computations were performed with Isodat 3.0 Gas Isotope Ratio MS Software (version 3.0; Thermo Fisher) and were based on comparisons with a standard reference gas (CO_2) of known isotopic composition that was repeatedly measured. The results from the analysis are reported in ‰ relative to an international standard (V-PDB). Replicate measurements of each sample and a mixture of fatty acid methyl esters (FAMEs) with $\delta^{13}C$ values traceable to international standards were used to determine the instrument precision (<0.3‰) and accuracy (<0.5‰). The values were also corrected subsequent to analysis to account for the methylation of the carboxyl group that occurs during acid extraction. The corrections were based on comparisons with a standard mixture of $C_{16:0}$ and $C_{18:0}$ fatty acids of known isotopic composition processed in each batch as a sample.

Supplementary Table S1: List of samples selected for lipid analysis (GC-MS, GC-c-IRMS) and bulk isotope characteristics of charred deposits (EA-IRMS).

Sample	Site	Type	Phase	Conc. (µg/g)	Compound detected	$\delta^{13}C$ C16:0 (‰)	$\delta^{13}C$ C18:0 (‰)	%C	$\delta^{13}C$ (‰)	%N	$\delta^{15}N$ (‰)	C:N
82	Slavnaya 4	ch[1]	EN	131	SFA($C_{14:0-18:0}$), UFA ($C_{16:1,18:1}$), DC(C_9),			40.09	-22.19	5.39	16.96	2.76
83	Slavnaya 4	ch[1]	EN	119	SFA($C_{14:0-18:0}$), UFA($C_{18:1}$), DC(C_{9-11}), APAA($C_{18,20tr}$), pri, phy			29.62	-19.40	4.69	18.55	1.86
84	Slavnaya 4	powder	EN	16	SFA($C_{14:0-26:0}$), DC(C_{8-10}), br, pri, phy, chol							
85	Slavnaya 4	powder	EN	36	SFA($C_{14:0-24:0}$), br, chol	-22.34	-22.48					
86	Slavnaya 4	ch[1]	EN	65	SFA($C_{14:0-30:0}$), UFA($C_{16:1,18:1}$), DC(C_9), pri, phy			20.82	-25.37	1.83	7.82	3.11
87	Slavnaya 4	powder	EN	27	SFA($C_{14:0-30:0}$), UFA($C_{16:1,18:1,20:1,22:1}$), br, pri, phy, chol	-20.11	-17.70					
88	Slavnaya 4	ch[1]	EN	78	SFA($C_{14:0-18:0}$), UFA($C_{16:1,18:1}$), DC(C_9)			29.96	-24.19	3.42	10.60	3.30
89	Slavnaya 4	ch[1]	EN	186	SFA($C_{14:0-18:0}$), UFA($C_{16:1,18:1}$), DC(C_9), pri			45.53	-24.47	4.36	13.06	4.07
90	Slavnaya 4	ch[1]	EN	211	SFA($C_{14:0-18:0}$), UFA($C_{16:1,18:1}$), DC(C_9), APAA(C_{18})			41.75	-20.14	6.03	18.39	2.65
91	Slavnaya 4	ch[1]	EN	177	SFA($C_{14:0-18:0}$), UFA($C_{16:1,18:1}$), DC(C_{8-13}), APAA(C_{16-22}), tmtd, pri, phy			62.10	-21.00	8.71	18.63	3.89

92	Slavnaya 4	ch[1,2]	EN	180	SFA($C_{14:0-26:0}$), UFA($C_{18:1}$), DC(C_{9-13}), APAA(C_{18-22}), pri, phy			39.31	-21.36	5.70	16.56	2.77
93	Slavnaya 4	ch[1]	EN	118	SFA($C_{14:0-30:0}$), UFA($C_{18:1}$), DC(C_{8-13}), APAA(C_{18-20}), tmtd, pri, phy			25.47	-23.40	2.54	12.74	2.33
94	Slavnaya 4	powder	EN	0	SFA($C_{16:0-18:0}$), UFA($C_{18:1}$), phy							
95	Slavnaya 4	powder	EN	5	SFA($C_{16:0-28:0}$), UFA($C_{18:1}$), phy							
96	Slavnaya 4	powder	EN	126	SFA($C_{14:0-30:0}$), UFA($C_{18:1,20:1,22:1}$), APAA(C_{18-22}), br, tmtd, pri, phy	-20.20	-19.14					
97	Slavnaya 4	powder	EN	8	SFA($C_{14:0-28:0}$), UFA($C_{18:1}$), br, phy							
98	Slavnaya 4	ch[2]	EN					34.29	-21.74	4.58	17.46	2.29
99	Slavnaya 4	ch[1]	EN	172	SFA($C_{12:0-18:0}$), UFA($C_{18:1}$), DC(C_9), tmtd, pri, phy			42.43	-24.65	3.64	13.26	3.73
100	Slavnaya 4	powder	EN	4	SFA(trace)							
101	Slavnaya 4	ch[1]	EN	189	SFA($C_{14:0-26:0}$), UFA($C_{18:1}$), DC(C_{9-13}), APAA(C_{18-22}), pri, phy			29.04	-23.37	3.66	13.82	2.45
101e	Slavnaya 4	ch.e[1]	EN	108	SFA($C_{14:0-18:0}$), UFA($C_{16:1,18:1}$), DC(C_9)							
102	Slavnaya 5	powder	EN	6	SFA($C_{14:0-18:0}$), UFA($C_{16:1,18:1}$), br							
103	Slavnaya 5	powder	EN	46	SFA($C_{14:0-26:0}$), UFA($C_{16:1,18:1,20:1,22:1}$), br, pri, phy, chol, terp(DT)	-18.95	-18.06					

104	Slavnaya 5	powder	EN	38	SFA($C_{14:0-18:0}$), UFA($C_{18:1}$), br, pri, phy	-23.32	-22.83					
106	Slavnaya 4	powder	MN	22	SFA($C_{14:0-18:0}$), phy							
107	Slavnaya 4	ch[1,2]	MN	936	SFA($C_{14:0-30:0}$), UFA($C_{16:1,18:1}$), DC(C_{7-13}), APAA(C_{18-22}), tmtd, pri, phy, chol			25.37	-23.21	3.01	13.52	2.19
108	Slavnaya 4	powder	MN	161	SFA($C_{14:0-28:0}$), UFA($C_{18:1,20:1,22:1}$), APAA(C_{18-22}), br, tmtd, pri, phy, chol	-23.45	-23.53					
109	Slavnaya 4	powder	MN	314	SFA($C_{14:0-18:0}$), br, tmtd, pri, phy, chol, terp(TT)	-24.40	-23.13					
110	Slavnaya 4	powder	MN	620	SFA($C_{14:0-28:0}$), UFA($C_{16:1,18:1,20:1,22:1}$), DC(C_9), APAA(C_{18-22}), br, tmtd, pri, phy, chol, terp(DT)	-23.13	-23.85					
111	Slavnaya 4	powder	MN	23	SFA($C_{14:0-18:0}$), UFA($C_{16:1}$), br, pri, phy, chol							
112	Slavnaya 4	powder	MN	67	SFA($C_{14:0-18:0}$), UFA($C_{18:1}$), APAA(C_{18}), br, tmtd, pri, phy	-23.16	-23.32					
113	Slavnaya 4	powder	MN	8	SFA($C_{14:0-26:0}$), UFA($C_{18:1}$), br, phy, chol							
114	Slavnaya 4	powder	MN	200	SFA($C_{14:0-20:0}$), UFA($C_{18:1,20:1}$), APAA(C_{18}), br, tmtd, pri, phy, chol	-23.80	-22.61					
115	Slavnaya 4	ch[1,2]	MN	1504	SFA($C_{14:0-18:0}$), UFA($C_{16:1,18:1}$), DC(C_{7-13}), APAA(C_{16-22}), tmtd, pri, phy, chol			43.33	-23.95	3.79	12.73	3.97
116	Chaivo 6	powder	MN	23	SFA($C_{12:0-24:0}$), UFA($C_{16:1,18:}$1), br, chol, phyto(b-sito)							
117	Chaivo 6	powder	MN	3	SFA($C_{14:0-18:0}$), UFA($C_{18:1}$), phy							

118	Chaivo 6	powder	MN	10	SFA($C_{14:0-18:0}$), UFA($C_{18:1}$), br		
119	Chaivo 6	powder	MN	36	SFA($C_{12:0-26:0}$), UFA($C_{16:1,18:1,20:1,22:1}$), DC(C_{7-12}), br, pri, phy, chol, phyto(b-sito)	-23.20	-22.06
120	Chaivo 6	powder	MN	18	SFA($C_{14:0-26:0}$), UFA($C_{18:1}$), DC(C_{8-12}), br, pri, phy, chol		
121	Chaivo 6	powder	MN	22	SFA($C_{12:0-24:0}$), UFA($C_{16:1,18:1}$), DC(C_{8-11}), br		
122	Chaivo 6	powder	MN	6	SFA($C_{16:0-18:0}$), UFA($C_{18:1}$), phy		
123	Chaivo 6	powder	MN	18	SFA($C_{12:0-24:0}$), UFA($C_{16:1,18:1}$), DC(C_{9-10}), br, chol		

Internal **(**ch) and external **(**ch**.**e) charred foodcrusts were analysed by acid-methanol extraction[1], alkali saponification[2], or both[1,2]. ($C_{n:x}$) - carboxylic acids with carbon length n and number of unsaturations x, SFA – saturated fatty acid, UFA – unsaturated fatty acids, DC - α,ω-dicarboxylic acids, APAA - ω-(o-alkylphenyl) alkanoic acids, br -branched chain acids dominated by *iso* and *anteiso* C_{15} and C_{17}, tmtd - 4,8,12-trimethyltridecanoic acid, pri – pristanic acid, phy – phytanic acid, chol – cholesterol or derivative, terp – indicate the presence of one or several terpenes, phyto(b-sito) – β-sitosterol.