Supplementary Material: Lewis et al., δ18O-inferred salinity from *Littorina littorea* (L.) shells in a Danish shell midden at the Mesolithic-Neolithic transition

Calculation of quantitative winter salinity estimates

Step 1. Determination of winter maximum $\delta^{18}O$ for cycle closest to the apex of the sub-fossil *Littorina littorea* (L.) shells (cycles with labelled winter maximum presented in Fig. 2, main text; see also Table S1).

Shell ID	First (clear) winter maximum $\delta^{18}O$ (VPDB)*	Conversion to SMOW**	Salinity (psu)†
NXA	1.15	-2.96	25.5
L7-1	1.24	-2.84	25.8
L7-2	1.57	-2.61	26.8
L7	1.39	-2.72	26.2
L4-1	-0.59	-4.33	20.1
L4-2	-0.77	-4.46	19.4
L4	1.59	-2.57	27.1
OEM	1.98	-2.26	28.2

Table S1. Summary of calculations for quantitative inference of winter salinity outlined in steps 1-3. *See Fig. 4, main text (W1), **determined in Fig. S1, †determined in Fig. S2.

Step 2. Conversion from $\delta^{18}O_{shell}$ (in VPDB) to $\delta^{18}O_{water}$ (in SMOW) at 3.7°C based on the modern Limfjord mixing line and relationship between temperature and salinity in the modern *L. littorea* shells from the Limfjord (northern Denmark) determined by Burman and Schmitz (2005) (Fig. S1).

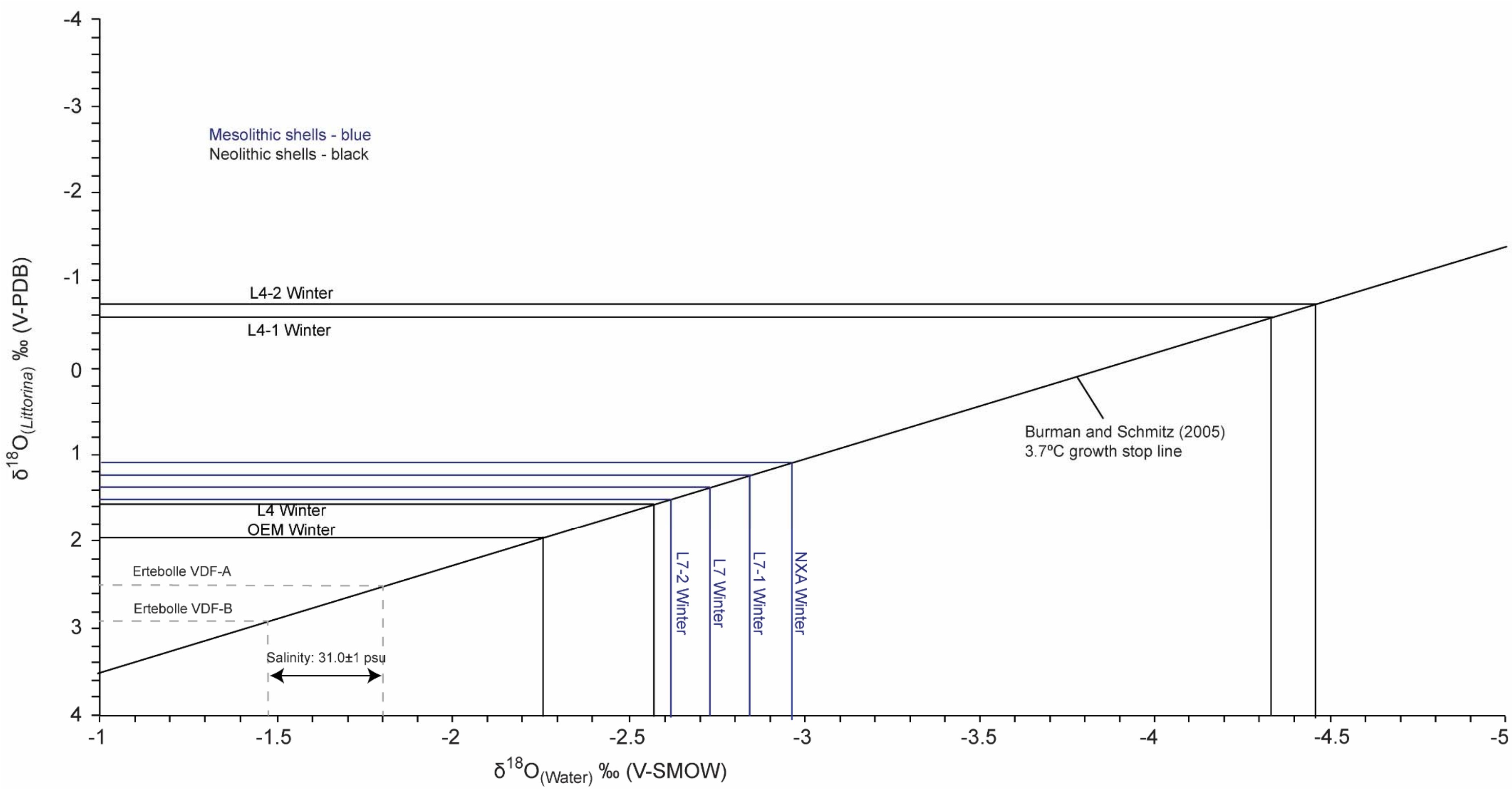


Figure S1. Plot showing Step 2, i.e. the conversion of the Norsminde $\delta^{18}O_{shell}$ sub-fossil winter maxima (in VPDB) to $\delta^{18}O_{water}$ (in SMOW) at the 3.7±1°C growth stop isotherm. This conversion is based on the modern Limfjord mixing line and relationship between temperature and salinity in the modern *Littorina littorea* shells from the Limfjord (northern Denmark) as determined by Burman and Schmitz (2005) (Fig S1). The sub-fossil conversion for the shells from the Late Mesolithic Ertebølle midden (from Burman and Schmitz, 2005) are also included for comparison.

Step 3. Conversion of $\delta^{18}O_{water}$ (in SMOW) to practical salinity units (psu) using the modern relationship between salinity and $\delta^{18}O$ (‰, SMOW) for the modern Limfjord water samples (Fig. S2).

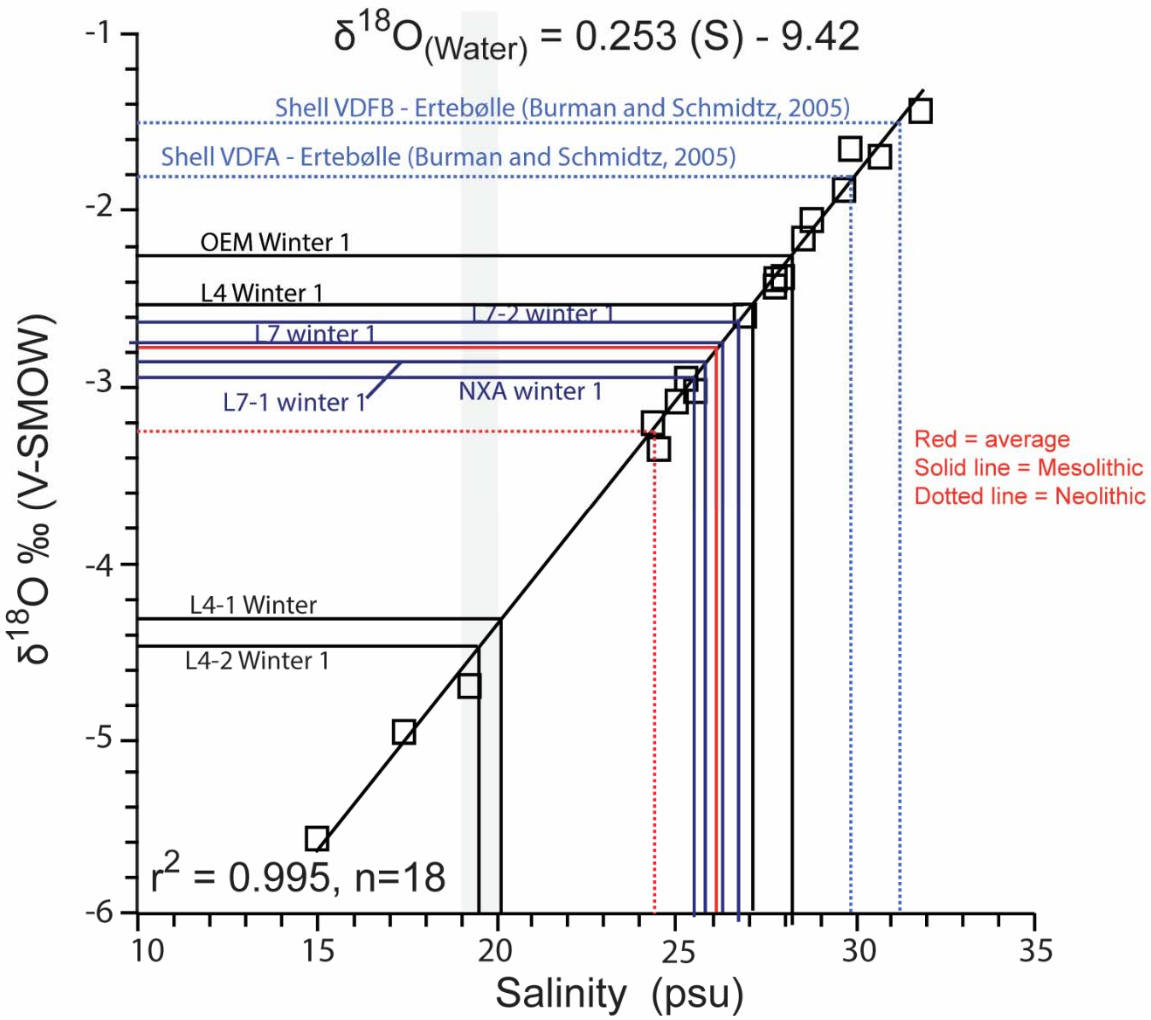


Figure S2. Modern relationship between salinity and $\delta^{18}O$ (‰, SMOW) for the modern Limfjord water samples taken along a salinity gradient from Struer (average salinity=32 psu) in the western Limfjord to Klitgård (average salinity=20.2 psu) in the east. Modern water samples analysed by Burman and Schmitz (2005). A linear correlation was observed (shown here by the Limfjord mixing line) yielding a freshwater end member of –9.4 ‰ $\delta^{18}O$ and an increase of +0.253 ‰ for each salinity unit. Following conversion of fossil shell winter $\delta^{18}O$ in (VPDB) maxima to $\delta^{18}O$ SMOW (step 2, Fig. S1), the salinity (at the 3.7°C growth stop) for the Mesolithic and Neolithic sub-fossil shells was estimated using this relationship (see also Table S1). Source: Figure modified from Burman and Schmitz (2005).

δ^{13}C

Overall δ^{13}C values range between –9‰ and +3.2‰, with early Neolithic shells exhibiting this whole range, but with values only falling between ~–2.3‰ to +1.6‰ to in shells from the Late Mesolithic (and generally above –1‰, with the exception of shell L7–1). Even within the Neolithic shells, values of below –1‰ are only found in two of the four individuals (L4–1 and L4–2). Some shells exhibit sinusoidal cycles in the δ^{13}C data (including L7–1, L4, L4–1 and L4–2), and in some cases at a similar wavelength to the δ^{18}O data (particularly L4–1 and L4–2; Fig. S3). However, shells L7, L7–2 and NXA from the late Mesolithic and shell OEN from the Early Neolithic, show more variability over a narrow range.

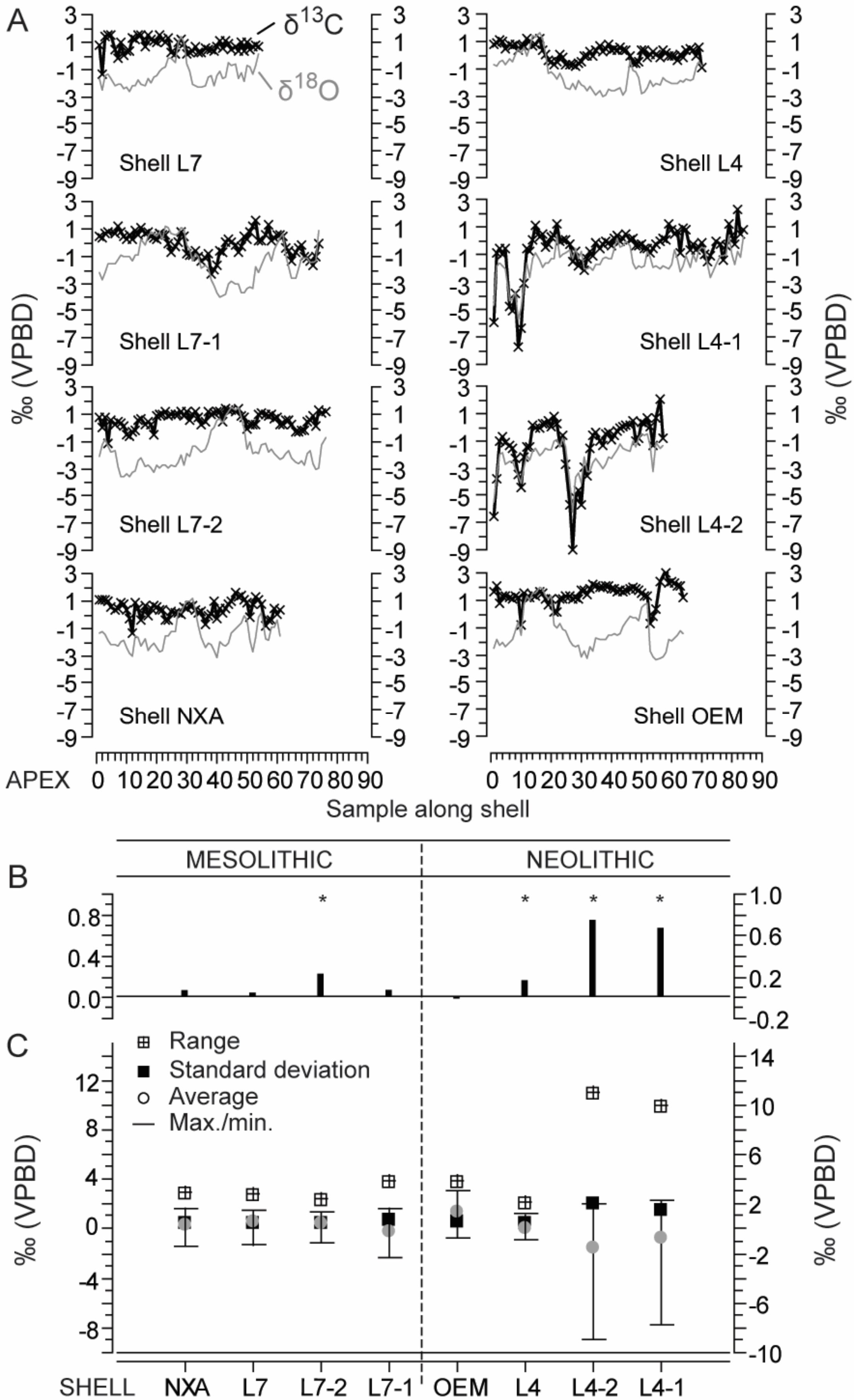
δ^{13}C data and seasonal trends

In all but two shells (i.e. L4–1 and L4–2; discussed below), the δ^{13}C variation over the entire profile falls between –2.3 and +3.2 ‰, suggesting that carbon source and utilisation is relatively consistent in each shell over its life span and between shells over the study period. These relatively high δ^{13}C values suggest that all Mesolithic and two of the Neolithic specimens primarily utilise dissolved inorganic carbon, directly from the ambient water (Fritz and Poplawski, 1974). Aquatic molluscs are known to build their shells primarily from ambient dissolved inorganic carbon (DIC) rather than respired CO_2 (McConnaughey and Gillikin, 2008). DIC yields high δ^{13}C (−3 and +3‰; Leng and Marshall, 2004), whilst dietary particulate algae and plant debris fall between −10 and −30‰ Meyers and Teranes, 2001. The range of δ^{13}C of the sub–fossil shells presented here yield similar δ^{13}C values to both the modern and sub–fossil shells sampled by Burman and Schmitz (2005), implying that source carbon for shell construction is largely consistent through time and across Danish coastal waters.

δ^{13}C vs. δ^{18}O

The relationship between δ^{13}C vs. δ^{18}O was compared for each shell and evaluated using regression analysis (Fig. S2; Table 1, main text). As indicated above some shells exhibit covariation between δ^{13}C vs. δ^{18}O, particularly the Early Neolithic–sourced shells L4–1 and L4–2 ($r=>0.8$, $r^2=>0.65$ and significant correlation; Fig S3 and Table 1, main text). In these two shells, a strong positive correlation exists between δ^{18}O and δ^{13}C, suggesting some seasonal shift in carbon uptake. Whilst the other shells may show some (mostly positive) correlation over part of their shell profile (Fig. S3), the magnitude of change is much smaller and the relationship is not consistent and therefore mostly falls below the significance level ($p = <0.01$).

With the exception of shells L4–1 and L4–2, there is no strong seasonal shift in δ^{13}C (when compared against the annual cycles identifiable in the δ^{18}O profile), suggesting no change in carbon source occurs over an annual cycle. However, in the Early Neolithic shells, L4–1 has high δ^{13}C values in the winter (compared against δ^{18}O profile), suggesting predominant utilisation of inorganic carbon in winter months, but in several summers (i.e. one in L4–1 and 2 in L4–2; Fig. S3) a substantial decrease in δ^{13}C occurs (values falling to <–5‰), indicating increased utilisation of respired CO_2 from dietary intake, in addition to DIC.



A
B
C
δ13C
δ18O
Shell L7
Shell L7-1
Shell L7-2
Shell NXA
Shell L4
Shell L4-1
Shell L4-2
Shell OEM
‰ (VPBD)
APEX
Sample along shell
MESOLITHIC
NEOLITHIC
Range
Standard deviation
Average
Max./min.
SHELL
NXA
L7
L7-2
L7-1
OEM
L4
L4-2
L4-1

Figure S3. A. Temporal (i.e. interannual) profiles of $\delta^{13}C$ (black symbols/lines) from the *Littorina littorea* shells analysed in this study. Isotope data are plotted against sample number (on x–axis), starting from the apex (i.e. youngest part of the shell=1) and following the direction of growth round the spiral towards to the outer lip. $\delta^{18}O$ data are also plotted (grey lines) to show any covariation between these two isotopes over each shell profiles (see also B; see main text for interpretation of $\delta^{18}O$ data). B. $\delta^{13}C$ vs. $\delta^{18}O$ regression statistics (r^2: black bars) for for each shell. Asterisks = statistically significant ($p<0.01$). C. $\delta^{13}C_{shell}$– isotope metrics including range, standard deviation, maximum, minimum and average (see also Table 1, main text).

References

Burman J and Schmitz B. (2005) Periwinkle (*Littorina littorea*) intrashell delta $\delta^{18}O$ and delta $\delta^{13}C$ records from the mid-Holocene Limfjord region, Denmark: a new high-resolution palaeoenvironmental proxy approach. *The Holocene* 15: 567-575.

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