

1 Title: Freshwater mussel (*Unio pictorum*) shells reveal hydrological
2 and environmental change from 1300 BC to the present day

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12 RUNNING TITLE: Growth rates in ancient and modern mussels

ABSTRACT

Preserved biological communities can provide baseline data about the historical ecosystems and environmental conditions that preceded recent anthropogenic alteration. Freshwater mussel shells show particularly good preservation, and the shell assemblages commonly found during archaeological excavations can offer insights into past ecosystems. We studied assemblages of *Unio pictorum* mussel shells from paleochannel silts associated with the Late Bronze Age site of Must Farm in eastern England (c. 850 BC), on an ancient tributary of the modern-day River Nene. We compared archaeological shells from two sediment horizons (broadly 1300 – 700 BC) to live individuals collected from two analogous sites on the present-day Nene. Size and growth rate, interannual growth variability, and stable isotope ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) composition were compared between the populations. Size and the von Bertalanffy growth parameter L_{∞} differed among all four populations. Mean lengths and L_{∞} were higher in the two modern populations (mean lengths $77.3 \pm \text{SE } 0.8$ mm and $73.8 \pm \text{SE } 1.1$ mm, L_{∞} 91.8 ± 5.4 mm and 79.0 ± 8.1 mm) than the ancient populations (mean lengths $58.1 \pm \text{SE } 1.6$ mm and $68.4 \pm \text{SE } 0.9$ mm; L_{∞} 71.5 ± 16.9 mm and 76.8 ± 6.2 mm). Modern individuals also showed greater variation in age-corrected year-to-year growth. $\delta^{13}\text{C}$ was lower in modern shells (-11.8‰ for modern shells, -9.03‰ and -9.02‰ for ancient shell populations), potentially reflecting altered hydrological and nutrient regimes. $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ were positively correlated for all but one sampled ancient shell, but not modern shells. These results reflect changes in local environmental conditions, particularly the transition from a shallow, slow-flowing tributary to a deeper, canalised river with faster flow, as well as effects of anthropogenic nutrient enrichment. The findings demonstrate the importance of long-term data in studying anthropogenic ecosystem alteration and avoiding shifting baseline syndrome in conservation planning.

KEYWORDS

Paleoecology, growth rate, stable isotope, nutrient enrichment, baseline, flow

1. INTRODUCTION

As the global degradation of ecosystems progresses as a result of long-acting anthropogenic ecosystem alteration, constructing an accurate representation of intact, non-degraded ecosystems becomes increasingly difficult. This can lead to so-called ‘shifting baseline syndrome’ (Pauly, 1995), whereby as ecosystem degradation progresses, perceptions of what an intact ecosystem looks like are also progressively degraded, in relation to conservation and restoration efforts. To avoid this, identifying accurate and reliable sources of information about historical ecosystems is an important step in setting conservation targets and developing interventions (Grace et al., 2019).

Freshwater mussels (Bivalvia: Unionida) are globally widespread, yet they are among the most threatened taxa. The IUCN lists 45% of species as globally Extinct, Threatened or Near Threatened (Lopes-Lima et al., 2018), and many other species have undergone local declines or extirpations (e.g. Lopes-Lima et al., 2023; Nakamura et al., 2023). These declines are closely linked to anthropogenic habitat disturbance and degradation, including habitat modification, pollution, and climate change (Lopes-Lima et al., 2017), and have been occurring for at least the past hundred years (Anthony & Downing, 2001). Due to this long history of degradation in mussel communities, it can be difficult to know what intact, pre-disturbance mussel communities may have looked like (for example, species composition and ranges, population density, age structure and individual body size) (Hornbach et al., 2018).

Freshwater mussel shells consist of nacreous and prismatic layers of aragonitic calcium carbonate with a proteinaceous outer layer called the periostracum (Checa, 2000). They are often well-preserved in sediments and are frequently found in archaeological excavations (Peacock, 2000; Randklev et al., 2010; Wolverson et al., 2010; Pickard et al., 2017). Studying these shells can provide valuable information about mussel communities in the pre-Industrial era, prior to significant and

intensive human alteration of habitats. These assemblages could therefore offer a baseline for conservation efforts. It should be noted that some archaeological shell deposits represent middens of shells harvested by humans, and were therefore subject to at least local human influence (Peacock, 2000).

In addition to representing historical mussel communities, archaeological shell assemblages can also offer information on environmental conditions during mussels' lifetimes. Comparing ancient shell assemblages to modern individuals and communities sampled from analogous locations can provide information on environmental change over time, although disentangling the many potential contributing factors can be challenging. Consideration should also be given to the effects of sampling bias and differential preservation (Peacock, 2000; Wolverton et al., 2010) and the possibility that shell deposits may have been assembled over long timescales (Nielsen & Helama, 2021).

Species identity can contribute to environmental reconstruction, since the presence of species with particular tolerances can provide information on environmental conditions (Pickard et al., 2017) such as water clarity or turbidity and silt levels (e.g. Mitchell & Peacock, 2014; Mitchell et al., 2023), although the possibility for evolutionary changes in species tolerances between modern and historical contexts should be considered. There is a general trend towards lower species diversity and fewer ecologically sensitive species in modern compared with archaeological mussel assemblages (Randklev et al., 2010; Wolverton et al., 2010; Peacock et al., 2016; Mitchell, 2018; Mitchell et al., 2023), reflecting disturbance, degradation and environmental homogenisation across ecosystems.

Patterns of mussel growth can also provide information about past environments and populations (Pickard et al., 2017). Mussel growth continues throughout the individual's lifetime, and in temperate climates, a period of winter growth cessation when temperatures fall below approximately 12°C (Dettman et al., 1999) results in the formation of an annual ring, visible as a dark band on the shell. Although ring formation can also be triggered by disturbance, annual rings offer a

reasonably reliable way to reconstruct mussel growth rates (Rypel et al., 2008; Versteegh et al., 2009). Mussel size and growth rates are highly variable both within and between populations (Haag & Rypel, 2011; DuBose et al., 2022) and are sensitive to a range of environmental factors, including temperature (Schöne et al., 2004; Lundquist et al., 2019), food availability (Arter, 1989; Versteegh et al., 2010; Müller et al., 2021), and flow regime (Rypel et al., 2008; Haag & Rypel, 2011; Butitta et al., 2021). Biotic factors including competition, parasites, disease and predation can also influence variability (Meira et al., 2024). Many of these variables are influenced by anthropogenic environmental alteration, including anthropogenic eutrophication and climate change, with warming temperatures shown to influence shell growth (Kendall et al., 2010).

Finally, the isotopic composition of mussel shells can provide further environmental information. Calcium carbonate in shells allows analysis of stable $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ isotopes. Bivalve shell carbonate is precipitated in oxygen isotopic equilibrium with ambient water (Dettman et al., 1999), and shell $\delta^{18}\text{O}$ is dependent on both $\delta^{18}\text{O}$ of ambient water and temperature during shell formation. Ambient water $\delta^{18}\text{O}$ depends on the balance of evaporation and precipitation, with $\delta^{18}\text{O}$ enriched in bivalve shells formed during warm, dry periods (Lewis et al., 2017). Elevated $\delta^{18}\text{O}$ has been reported for modern compared to ancient shells (Van Plantinga & Grossman, 2018; Collins et al., 2020), suggested to reflect modern effects of drought and impoundment. In contrast, Bar-Yosef Mayer et al (2012) found higher $\delta^{18}\text{O}$ in ancient shells, possibly reflecting intense summer evaporation at the time of shell formation. Shell $\delta^{18}\text{O}$ can provide an accurate record of temperature (Kukolich & Dettman, 2021), so incremental samples from freshwater mussel shells generally show clear seasonality in $\delta^{18}\text{O}$ (Pfister et al., 2018). In addition, higher $\delta^{18}\text{O}$ is expected in mussels from lakes and shallow, low flow environments compared to flowing rivers (Vonhof et al., 2013; Apolinarska & Kurzawska, 2020), since longer residence time allows greater evaporation of ^{16}O .

$\delta^{13}\text{C}$ in freshwater mussel shell is largely dependent on the isotopic composition of ambient dissolved inorganic carbon (DIC). DIC $\delta^{13}\text{C}$ can vary with factors including precipitation (Schöll-Barna

et al., 2012), water depth and residence time (Apolinarska & Kurzawska, 2020), lake versus river habitat (Kaandorp et al., 2006; Vonhof et al., 2013), reproduction (Dettman et al., 1999), salinity (McConnaughey & Gillikin, 2008), and catchment vegetation (Kelemen et al., 2017). A generally smaller but variable contribution to shell $\delta^{13}\text{C}$ derives from metabolic carbon (Dettman et al., 1999; McConnaughey & Gillikin, 2008; Lewis et al., 2017), which decreases shell $\delta^{13}\text{C}$. This variable contribution can obscure environmental effects on shell $\delta^{13}\text{C}$ (Dettman et al., 1999; Kelemen et al., 2017). In addition, the combustion of fossil fuels in the post-Industrial era has led to an overall depletion of atmospheric $\delta^{13}\text{C}$ by approximately 2‰, known as the Suess effect (Keeling, 1979; Rubino et al., 2013). The extent and way in which this may affect aquatic and shell $\delta^{13}\text{C}$ is complex and poorly understood (Apolinarska & Kurzawska, 2020).

Stable isotope analysis has been used to examine changing climate (Peacock & Seltzer, 2008; Bar-Yosef Mayer et al., 2012; Schöll-Barna et al., 2012), habitat conditions (Vonhof et al., 2013; Van Plantinga & Grossman, 2018), food sources (Lewis et al., 2017) and trophic state (Fritts et al., 2017) in historical compared to modern mussel populations. When using archaeological shells for stable isotope analysis, it is important to test first for possible diagenetic alteration of the carbonate material being analysed to eliminate the possibility of post-shell formation changes to isotopic ratios.

We applied these analytical approaches to freshwater mussel shell assemblages from a Late Bronze Age archaeological site. The Must Farm pile-dwelling settlement was built on an ancient tributary of the present-day River Nene in the fens of eastern England. The modern-day River Nene and surrounding landscape has undergone significant alteration due to human activities, including extensive draining of the peat fens to expand land availability for agriculture. Attention towards restoration of this landscape has recently been increasing, including interventions such as peat rewetting. Given this long history of modification and the opportunities for restoration, this is a particularly relevant context to explore the use of mussels for studying past ecosystems and establishing ecological baselines for conservation.

Dated to c. 850 BC (Knight et al., 2024), the Late Bronze Age pile-dwelling settlement on the Nene consisted of several houses and numerous associated artefacts. The site appears to have been occupied for 9-12 months and then abandoned rapidly after a fire. During excavations, abundant freshwater shell deposits were found in the river silts underlying, and thus preceding, the construction horizon. Similarly abundant freshwater shells were also found in a silty river deposit overlying, and thus post-dating, the conflagration debris. The mussels in both sampled deposits are believed to have died naturally where they were found (Allen, 2010), rather than being resources collected by humans (c.f. Evans, 2015). We analysed the size, growth rates, interannual growth variability and stable isotope composition of shells from both deposits at Must Farm, and compared these with live mussels collected from two nearby sites on the present-day River Nene main and side channels. We also compared species composition between the modern and ancient sites.

We predicted that species composition would have changed, with the possible decline of the more ecologically sensitive species *Pseudanodonta complanata*. We expected that size and growth rates would be higher in modern compared with ancient mussels due to the effects of anthropogenic nutrient enrichment, which would be expected to increase algal growth and therefore food availability for mussels. The effects are likely to be particularly strong in our study area, which is now intensely cultivated and subject to anthropogenic eutrophication (Balbi, 2000). In addition, we expected that environmental change and associated increased variability would lead to more variability in year-to-year growth in modern populations compared with ancient ones. Linked to this, we expected to find differences in both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ reflecting altered environmental conditions and nutrient availability and more variation within samples from the same individual for modern mussels.

2. METHODS

2.1 Study sites

Ancient bivalve shells were collected during archaeological excavations of the Bronze Age pile-dwelling settlement of Must Farm, near Peterborough, UK (52°33'18"N, 0°10'39"W; Fig. 1). The site is located on a paleochannel tributary of the River Nene. The settlement has been dated to c. 850 BC, and the associated palaeochannel sediments overall span from the late 17th – late 16th centuries BC to the 1st century AD (Ballantyne et al., 2024; Tyers et al., 2024). The shells in this analysis were collected from two shell-rich silt deposits: one preceding the settlement and labelled 'Must Farm 1' (deeper and older context, 13th – early 9th centuries BC) and one post-dating the settlement labelled 'Must Farm 2' (shallower and more recent context, late 9th – 7th centuries BC). Shells were hand-collected from sediment samples processed by wet sieving over 4mm mesh. Only intact left valves were selected for analysis to avoid inadvertently sampling two valves from the same individual.

In total we retrieved and measured n = 35 mussels from Must Farm 1 and n = 59 mussels from Must Farm 2. All the intact mussels from Must Farm were *Unio pictorum* (Linnaeus, 1758). Fragments of *Pseudanodonta complanata* (Rossmässler, 1835), *Anodonta anatina* (Linnaeus, 1758) and suspected *Anodonta cygnea* (Linnaeus, 1758) and *Unio tumidus* (Philipsson, 1788) were also retrieved from samples but discarded for analysis since no complete intact shells were found.

Modern-day live mussels were collected from two sites on the current course of the River Nene (Fig. 1), as close as possible to the Must Farm site: a site on the main channel (52°34'32"N, 0°7'54"W; samples labelled 'River Nene') and King's Dyke, a side channel of the Nene (52°33'15"N, 0°10'19"W; samples labelled 'King's Dyke'). Both channels run through intensively cultivated land. Both sampling sites were directly downstream of the town of Peterborough and receive effluents from multiple urban sewage treatment plants.

Mussels were collected by hand in February 2019. In total we collected and measured n = 83 mussels from King's Dyke and n = 98 mussels from the River Nene. Mussels of species *U. pictorum*, *U. tumidus*, *A. anatina* and *A. cygnea* were present at King's Dyke, and *U. pictorum* and *A. anatina*

were present at the River Nene. In addition, the invasive zebra mussel *Dreissena polymorpha* (Pallas, 1769) was present at both sites, with some low-level fouling of unionids (no more than two zebra mussels per unionid). Only *U. pictorum* was used for analysis since this could be compared across all four populations. This species has previously been shown to form annual rings which can provide a record of seasonal temperature (Lundquist et al., 2019).

The difference in sampling methodology between the two time periods could lead to some differences in size distribution among the sampled individuals, since tactile searches can favour larger individuals (Vaughn et al., 1997). In contrast, mussels sampled from the archaeological assemblages should represent the full size range of the population, although size-related preservation differences might also be expected (Peacock, 2000; Wolverton et al., 2010). These potential biases could affect comparisons of length between the sites, but should not affect comparisons of growth patterns. Age distributions for all samples are presented in Figure 2.

Environmental data for the King's Dyke and River Nene sites were retrieved from the Environment Agency Water Quality Monitoring Archive for the period of the mussels' lifetimes for which data were available. For King's Dyke the Environment Agency sampling site used was 'King's Dyke Stanground Sluice' (52°33'37"N, 0°13'7"W) (Environment Agency, 2019a), located a Euclidean distance of 3.25km from our King's Dyke sampling point, and for the River Nene we used 'River Nene Peterborough Town Bridge' (52°34'6"N, 0°14'29"W) (Environment Agency, 2019b) monitoring point, located a Euclidean distance of 7.5km from our River Nene sampling point. Water quality samples for King's Dyke were taken between 30 May 2006 and 7 May 2014, and samples for River Nene were taken from 19 January 2006 to 19 April 2017 (regular monitoring stopped at both sites some time before data collection for our study, so more recent data were not available). Variables relevant to freshwater mussel ecology for which data were available for the full duration of the monitoring period were alkalinity, oxidised nitrogen, dissolved oxygen, orthophosphate, and temperature. Measurements were irregular, with 1-2 samples per month for most variables. We retrieved n = 100

complete and partially complete water quality samples for King's Dyke, and $n = 138$ complete and partially complete samples for the River Nene. A summary of these abiotic parameters is given in Table 1. The sites differed significantly only in alkalinity, which was higher in the River Nene ($t_{(124)} = 3.59$, Holm-adjusted $p = 0.003$).

The paleochannel tributary was shallow and slow-flowing (Ballantyne et al., 2024) with seasonal variation in flow (French, 2024). It underwent periods of drying, and at times may have been temporarily detached from the main flow. In contrast, both King's Dyke and the River Nene exhibit deep water (2-3m) with continuous year-round flow. Total phosphorus (TP) modelling has also been conducted for the Must Farm palaeochannel deposits, based upon the ancient diatom and chironomid populations (Cameron, 2024; Langdon, 2024). The chironomids show that pre-settlement 'Must Farm 1' had TP of $72\text{--}74 \mu\text{g L}^{-1}$ while the post-settlement 'Must Farm 2' had TP of $35\text{--}65 \mu\text{g L}^{-1}$. While the diatom modelling generates slightly different values depending on the regression method used, the same trend in values is present. In both cases, clearly eutrophic conditions were present, and the brevity of the Late Bronze Age settlement therefore did not have a lasting impact on water nutrient levels.

Table 1: Abiotic variables for sampling sites. Present-day data are taken from the Environment Agency's water quality monitoring archive from 30 May 2006 to 7 May 2014 for King's Dyke ($n = 100$) and from 19 January 2006 to 19 April 2017 for the River Nene ($n = 138$). Archaeological values are estimated from associated populations of diatoms, chironomid larvae and ostracod valves at Must Farm (Cameron, 2024; Langdon, 2024; Timberlake, 2024).

Variable	Must Farm 1 (estimated)	Must Farm 2 (estimated)	King's Dyke (mean $\pm$ SE)	River Nene (mean $\pm$ SE)
pH	8	8	n.d.	n.d.
Alkalinity ($\text{mg L}^{-1} \text{CaCO}_3$)	n.d.	n.d.	183.35 ± 2.22	195.05 ± 2.39
Oxidised nitrogen (mg L^{-1})	n.d.	n.d.	6.18 ± 0.20	6.92 ± 0.21

Dissolved O ₂ (mg L ⁻¹)	6-8	6-8	10.50 ± 0.29	10.47 ± 0.22
Orthophosphate (mg L ⁻¹)	0.07	0.04-0.07	0.15 ± 0.01	0.18 ± 0.01
Water temperature (°C)	8-9	10	12.10 ± 0.55	12.26 ± 0.53

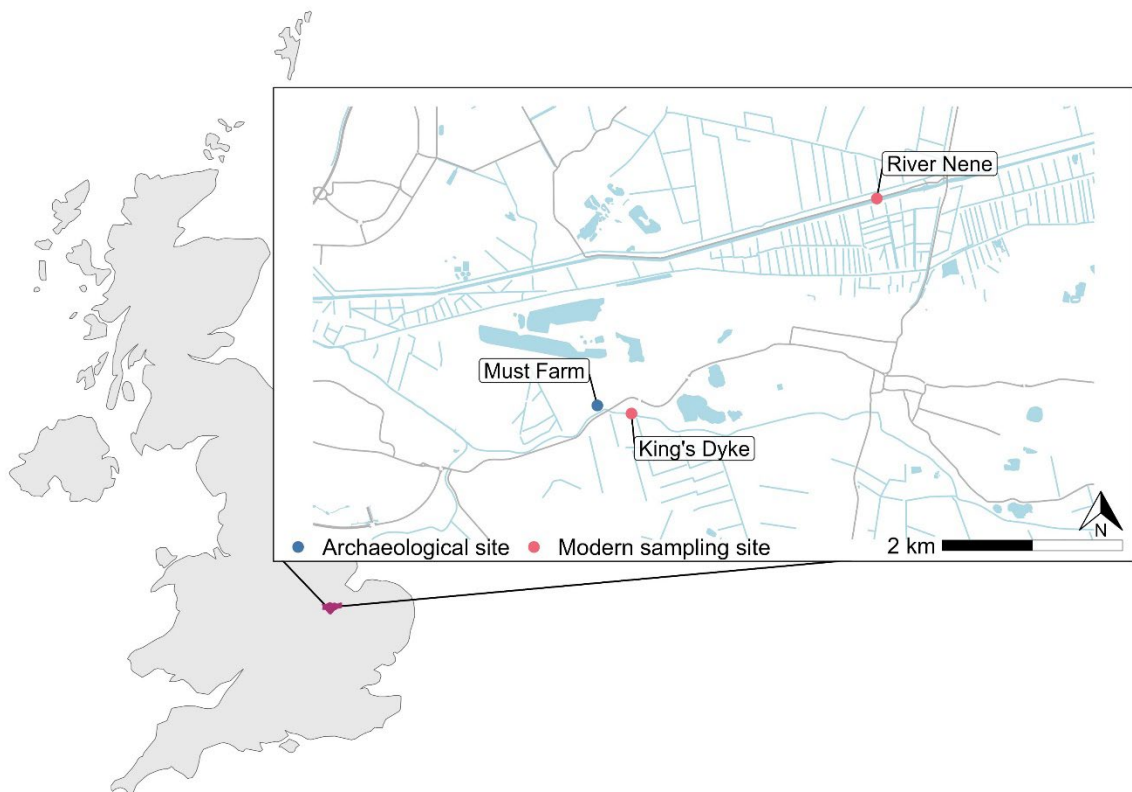


Figure 1: Map of sampling sites. The Peterborough region where sampling took place is marked on the larger map. Map created using data from [OpenStreetMap](https://www.openstreetmap.org/).

2.2 Data collection

We measured the length of each mussel along its longest (anterior-posterior) axis, as well as the length of each annual growth ring along the same axis, using Vernier callipers.

In addition, isotopic ratios for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ were analysed for shell samples from a subset of the sampled shells ($n = 3$ for each of Must Farm 1, Must Farm 2 and King's Dyke). We did not conduct isotopic analysis of shells from the River Nene site, since we expected that the difference in river size between this site and the paleochannel could lead to large site-based differences in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ which would be likely to obscure any effects of temporal change. Shells were first fixed in resin, and a thick section (approx. 3 mm) from each individual was cut from the umbo to ventral margin, at the widest (central) part of the shell. Powdered shell samples were then drilled in a series from umbo to ventral margin along the centre line of the shell section, so that periostracum was not sampled. Fourteen to 22 samples were drilled per shell to a depth of 1.5 mm, leaving a distance of approx. 2 mm between sampling points. Drilling was conducted using a Sherline mill (model 5410, Sherline Products, Inc. Vista, California 92081, USA) with a tungsten carbide drill bit of diameter 0.6 mm (H1 204 006, Komet Dental, Gebr. Brasseier GmbH & Co. KG, Trophagener Weg 25, 32657 Lemgo, Germany). A drilling speed of 70 ± 10 rpm was used to minimise heating and the potential for diagenetic alteration of isotopic ratios.

Samples from Must Farm were analysed using FTIR-ATR to test for the presence of calcite. A high calcite to aragonite ratio would indicate diagenetic alteration of isotope ratios in the period since shell formation, making isotope analysis uninformative. Analysis was carried out in the Charles McBurney Laboratory for Geoarchaeology at the University of Cambridge using a Thermo Nicolet iS5 spectrometer. Samples were measured using 64 reads per sample. Spectra for each sample were compared against a reference series of standard calcite-aragonite mixes, which were used to estimate the calcite-aragonite ratio in samples. Characteristic absorbance peaks in the ranges 690-720 cm^{-1} , 820-890 cm^{-1} , 1070-1095 cm^{-1} , 1200-1600 cm^{-1} , and 1760-1820 cm^{-1} were used in analysis, using methodology described by Loftus et al (2015). Only one sample, from Must Farm 1, was determined to have a calcite content of $>5\%$, and this was excluded from isotopic analysis. Following visual inspection of spectra, two further samples each from two shells from Must Farm 2 were excluded from further analysis due to irregularities.

The remaining Must Farm 1 & 2 samples and the King's Dyke samples were then sent for isotope analysis for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$. Analyses were performed at The Godwin Laboratory for Palaeoclimate Research, Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EQ, UK

Drilled powdered samples were transferred to exetainer vials and sealed with silicone rubber septa using a screw cap. The samples were flushed with CP grade helium then acidified with 104% orthophosphoric acid, left to react for 1 hour at 70 degrees Celsius and then analysed using a Thermo Gasbench preparation system attached to a Thermo Delta V Advantage mass spectrometer in continuous flow mode. Each run of samples was accompanied by 10 reference carbonates (Carrara Z) and 2 control samples (Fletton Clay). Carrara Z has been calibrated to VPDB using the international standard NBS19. The results are reported with reference to the international standard VPDB and the precision is better than ± 0.08 ppm for $\delta^{13}\text{C}$ and ± 0.10 ppm for $\delta^{18}\text{O}$.

All collected data are publicly available (Ollard et al., 2024).

2.3 Data analysis

All analyses were conducted in R v4.2.2 (R Core Team, 2022).

Growth curves were calculated using the von Bertalanffy growth model (VBGM; Eq. 1; von Bertalanffy, 1938), which has been shown to represent bivalve growth patterns (Hastie et al., 2000). This model includes three constants: L_{∞} is asymptotic length, K is the rate at which asymptotic length is reached, and t_0 relates to initial size (specifically, the age at which length is zero). Models were fitted to annual length-at-age data using the function *nls* in base R. 95% confidence intervals for growth curves were estimated by bootstrapping with 999 resamples for each population. Growth curves were compared between populations using likelihood ratio Kimura tests (Kimura, 1980; Nelson, 2019). We constructed a general VBGM in which all three coefficients (L_{∞} , K and t_0) varied among populations, and a set of further models in which each coefficient in turn was constrained, as

well as model where no coefficients varied among populations (Equations 2 – 6). We compared each constrained model against the general model using likelihood-ratio chi-square tests, where a value of $p < 0.05$ indicated that the constrained model fit the data significantly less well than the general model, and hence that the variable being tested varied significantly among populations. To conduct post-hoc pairwise comparisons, we then repeated the process with each pair of sites, applying a Holm correction for multiple comparisons to all chi-square test p-values.

$$L_t = L_{\infty}(1 - e^{-K(t-t_0)})$$

Von Bertalanffy growth model (VBGM) where t = age in years, L_t = length at age, and L_{∞} , K and t_0 are constants.

Equation 1.

$$L \sim L_{\infty(site)}(1 - e^{-K(site)(t-t_0(site))})$$

H_0 : All parameters vary between populations.

Equation 2.

$$L \sim L_{\infty}(1 - e^{-K(site)(t-t_0(site))})$$

H_1 : L_{∞} constant across populations.

Equation 3.

$$L \sim L_{\infty(site)}(1 - e^{-K(t-t_0(site))})$$

H_2 : K constant across populations.

Equation 4.

$$L \sim L_{\infty(site)}(1 - e^{-K(site)(t-t_0)})$$

H_3 : t_0 constant across populations.

Equation 5.

$$L_t = L_\infty(1 - e^{-K(t-t_0)})$$

H_4 : All parameters constant across populations.

Equation 6.

We also analysed within-mussel interannual growth variability in the length of annual growth increments (rather than lengths, to remove year-to-year non-independence). We fitted a negative exponential with a constant term (Eq. 7) to each population separately, again using the *nls* function, and calculated expected increments-at-age for each population using these fitted models. This served to detrend the data, since increments become smaller at older ages. We then calculated a Growth Index (GI) value for each increment for each individual, using Eq. 8. GI values therefore represented the extent of divergence of an individual growth increment at a particular age from the population-wide expected growth increment at that age. This also de-correlated increment variance from age. Finally, we calculated the standard deviation of GI (σ GI) values for each individual mussel to give a measure of within-individual interannual growth variability. A high σ GI value indicated greater variation in annual growth increments, since among increments there was a greater range of divergence from the population expected values; a small σ GI value indicated smaller interannual growth variation, and small values could be obtained even if individuals diverged significantly from population expected values, as long as this divergence was consistent between years, since this would result in consistently large or small GI values. σ GI values were log-transformed for subsequent analyses to meet assumptions of normality and homoscedasticity. We compared the effect of site and time (modern versus ancient) on log-transformed σ GI using two-way ANOVA.

$$I_t = ae^{-bt} + c$$

Negative exponential model for increment length at age, where I_t = increment length at age, t = age in years, and a , b and c are constants.

Equation 7.

$$GI_t = I_t/E_t$$

Method for calculating Growth Index values where GI_t = Growth Index at age, t = age in years, I_t = increment length at age, and E_t = expected increment length at age, using models fitted using Eq. 7.

Equation 8.

Isotope data were analysed using linear mixed models, using the packages *lme4* (Bates et al., 2015) for model construction, *lmerTest* (Zeileis & Hothorn, 2002) for model testing and *emmeans* (Lenth, 2024) for pairwise comparisons. Models were constructed with each isotope ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) separately as dependent variable, site (MF1, MF2 and KD) as the fixed effect and individual mussel as the random effect.

To test differences between sites in within-individual inter-sample variability for isotope data, we considered whether it was necessary to correct for ontogenetic effects, as we did for comparing growth rate variability. Evidence from the literature on possible ontogenetic effects on isotopic composition varies. A lack of correlation has been reported for both $\delta^{18}\text{O}$ (Kelemen et al., 2017) and $\delta^{13}\text{C}$ (Yoshimura et al., 2015; Kukolich & Dettman, 2021). Other studies have reported declining $\delta^{13}\text{C}$ with age (McConnaughey & Gillikin, 2008; Błażejowski et al., 2013; Kelemen et al., 2017), although Kaandorp et al (2006) found the opposite pattern. Błażejowski et al (2013) also reported a relationship between $\delta^{18}\text{O}$ and age. We tested for ontogenetic effects of age-isotope correlation for both oxygen and carbon isotopes using the Pearson's correlation test of isotopic ratio against sample number (using sample number as an approximate proxy for age, although since samples were evenly spaced across the shell younger ages were more highly represented in the pool of samples). One mussel (shell 8 from MF2; Pearson's coefficient = -0.705) showed a significant decrease in $\delta^{18}\text{O}$ with age, and a further one (shell 17 from KD; Pearson's coefficient = -0.485) showed a significant decrease in $\delta^{13}\text{C}$ with age; we did not consider that this warranted any overall correction for ontogenetic effects. To test within-individual variability among samples we therefore compared the

residuals, grouped by site, of a mixed model using site as a fixed effect and individual mussel as a random effect, using Levene's test of homoscedasticity. This tested whether variance of observations around the mean for each individual and site differed significantly by site.

3. RESULTS

3.1 Length and growth curves

Lengths differed significantly between all mussel populations ($F_{(3,246)} = 50.78$, $p < 0.001$, adjusted $R^2 = 0.375$; Fig. 2A). Pairwise comparisons showed significant differences between all site pairs ($p < 0.05$ for all pairwise comparisons). Modern mussels had larger size than ancient mussels. Mean length was greatest for the River Nene site ($77.3 \pm \text{SE } 0.8$ mm), followed by King's Dyke ($73.8 \pm \text{SE } 1.1$ mm), Must Farm 2 ($68.4 \pm \text{SE } 0.9$ mm) and Must Farm 1 ($58.1 \pm \text{SE } 1.6$ mm).

Growth curves also differed among all sites (models H0 vs H4; $X^2_{(9)} = 565.93$, $p < 0.001$; Fig 2B). The VBGF coefficient L_{∞} differed significantly between sites (models H0 vs H1; $X^2_{(3)} = 12.69$, $p = 0.005$). There were no significant differences between sites in the parameters K (models H0 vs H2; $X^2_{(3)} = 4.97$, $p = 0.174$) or t_0 (models H0 vs H3; $X^2_{(3)} = 3.11$, $p = 0.375$). Estimated VBGF parameters are reported in Table 2. There were significant differences for all pairwise site comparisons when comparing overall growth curves ($p < 0.001$ for all cases; Appendix S1). However, there were no significant differences in individual growth parameters ($p > 0.05$ for all cases; Appendix S1).

Fisher's exact test showed significant differences in age among populations ($p < 0.001$; Fig. 2C). However, the pattern of results did not coincide with differences in length among populations. Mean age was oldest for Must Farm 2 ($7.42 \pm \text{SE } 0.21$ years), followed by King's Dyke ($7.13 \pm \text{SE } 0.32$ years), River Nene ($6.66 \pm \text{SE } 0.18$ years) and Must Farm 1 ($5.57 \pm \text{SE } 0.20$ years).

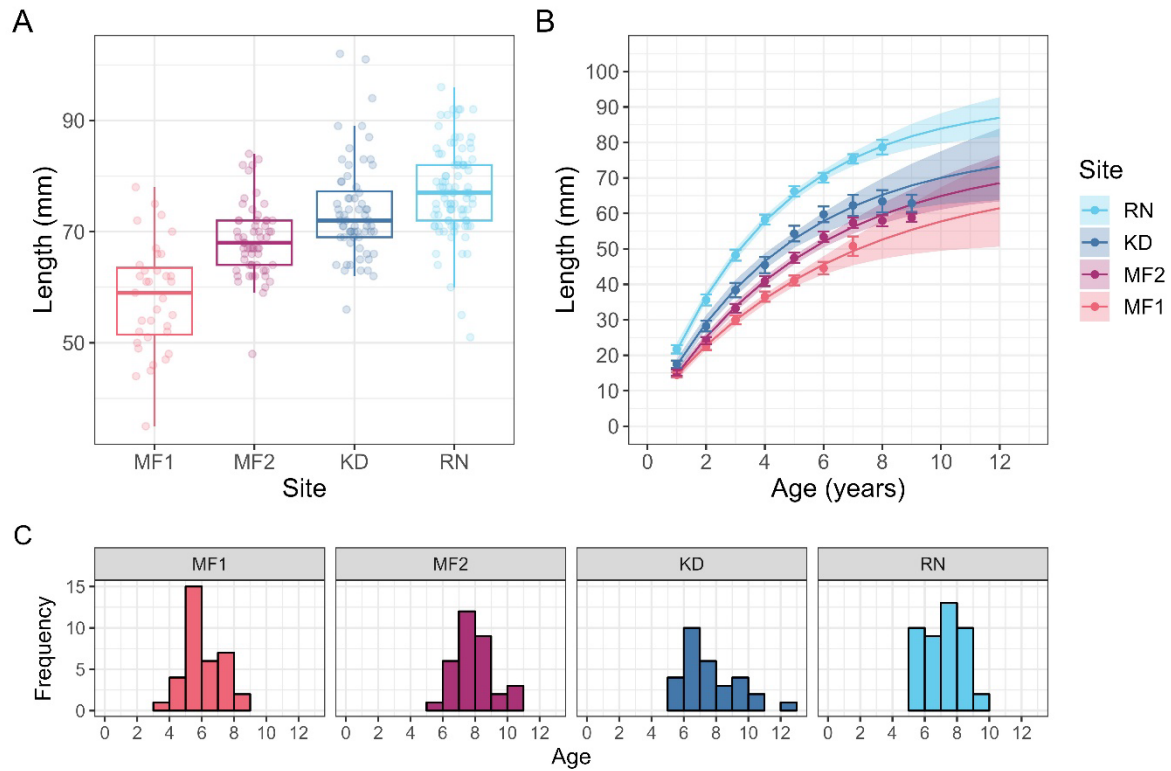


Figure 2: Length (A), fitted von Bertalanffy growth curves (B) and age-frequency plots (C) for each site. In 2A, the boxes show median and interquartile range, and the endpoints of the whiskers extend to 1.5 * IQR beyond the boxes. In 2B, growth curves are shown with bootstrapped 95% confidence intervals. Mean lengths-at-age with standard error bars are also plotted. MF1 = Must Farm 1; MF2 = Must Farm 2; KD = King's Dyke; RN = River Nene.

Table 2: Von Bertalanffy growth parameters for each population. L_{∞} = asymptotic length; K = rate at which asymptotic length is attained; t_0 = age at which length equals zero. RSE = residual standard error.

Site	L_{∞}	K	t_0	RSE _(df)
King's Dyke	79.0 ± 8.1	0.214 ± 0.047	-0.146 ± 0.17	5.15 _(3,206)
River Nene	91.8 ± 5.4	0.243 ± 0.034	-0.090 ± 0.129	6.29 _(3,288)
Must Farm 1	71.5 ± 16.9	0.158 ± 0.063	-0.426 ± 0.241	5.55 _(3,191)

Must Farm 2	76.8 ± 6.2	0.184 ± 0.031	-0.149 ± 0.144	5.52 _(3,239)
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3.2 Interannual growth variability

There were significant differences in interannual variability between modern and ancient mussels (Fig. 3). Using two-way ANOVA, time (modern versus ancient) was a significant (but weak) predictor of within-mussel interannual growth variability, measured as log (σ Growth Index) ($F_{(1,138)} = 7.93$, $p = 0.006$), but site was not ($F_{(2,138)} = 0.20$, $p = 0.818$); adjusted $R^2 = 0.036$.

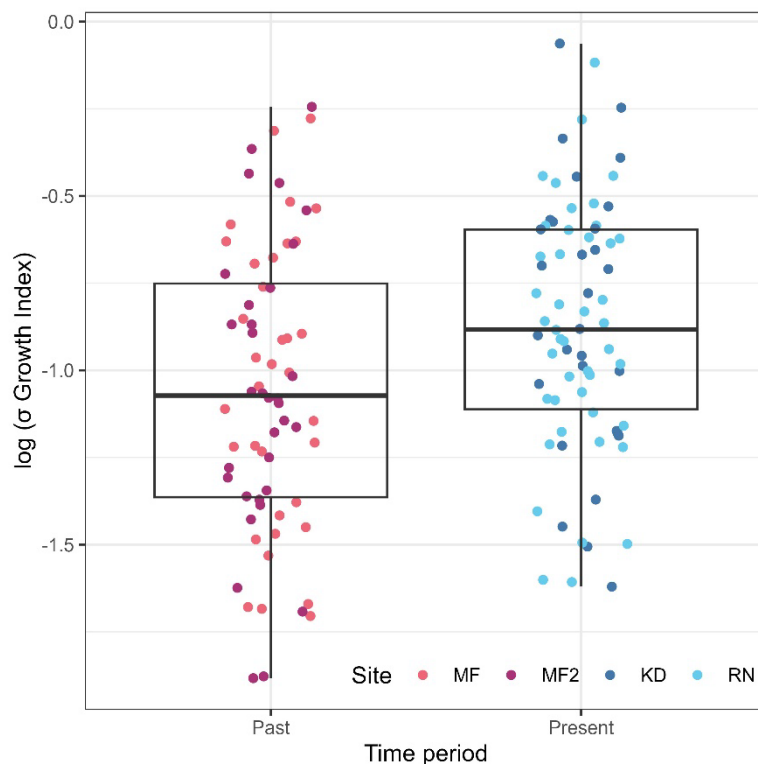


Figure 3: Within-mussel interannual variability in growth, measured as log (σ Growth Index).

3.3 $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotope analysis

For $\delta^{18}\text{O}$, site was a significant predictor of isotopic ratio in a mixed model that included the individual mussel from which the sample was taken as a random effect ($\chi^2_{(2)} = 7.18$, $p = 0.028$; Fig. 4).

There were no significant pairwise differences among sites ($p > 0.05$ for all pairwise comparisons). Similarly, for $\delta^{13}\text{C}$ site was also a significant predictor of isotopic ratio in mixed models that included individual mussel as a random effect ($\chi^2_{(2)} = 21.02$, $p < 0.001$). Pairwise comparison showed that mussels from MF1 and MF2 were not significantly different from one another, but that mussels from both sites differed significantly from KD mussels ($p = 0.998$ for MF1-MF2; $p < 0.001$ for both MF1-KD and MF2-KD).

Unlike the results for growth patterns (Fig. 3), there were no significant differences in inter-sample variability – i.e. the extent to which samples from the same individual differed in their isotopic composition – among sites for either $\delta^{18}\text{O}$ ($F_{(2,164)} = 0.881$, $p = 0.416$) or $\delta^{13}\text{C}$ ($F_{(2,164)} = 1.74$, $p = 0.178$).

$\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ isotope ratios showed significant positive correlations for five out of the six ancient mussels (Table 3, shells A-E), indicating in-phase variation (values for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ increase and decrease in synchrony). The sixth ancient mussel (Table 3, shell F), from Must Farm 2, showed no significant correlation. In contrast, among the modern samples, two out of three mussels (Table 3, shells G and H) showed no significant correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, while the third (Table 3, shell I) showed significant negative correlation, indicating out-of-phase variation between the two isotopic ratios, with one increasing when the other decreases and vice versa.

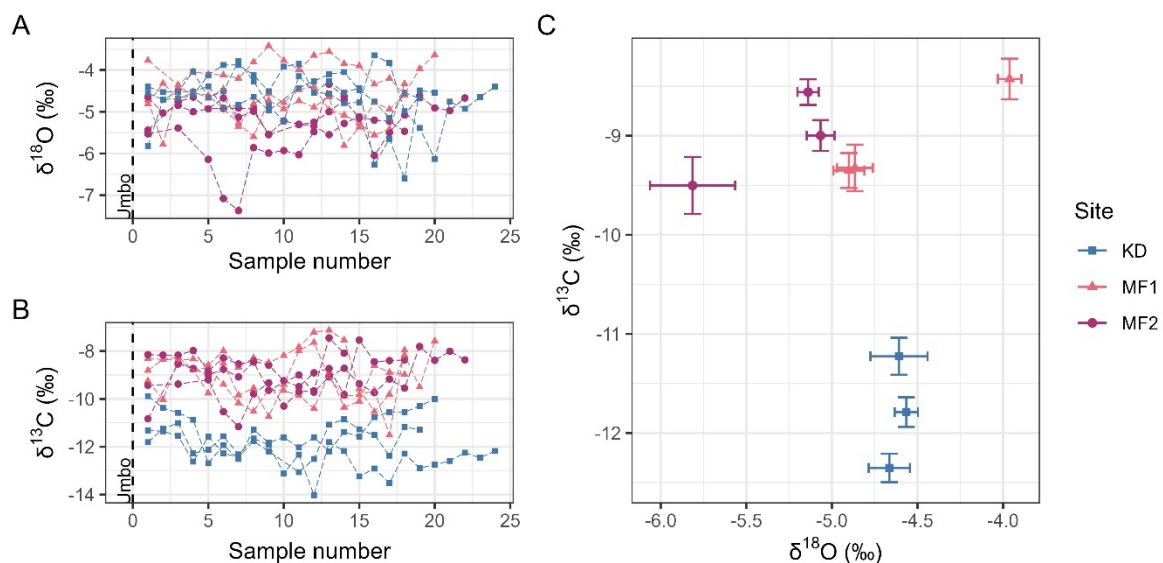


Figure 4: Isotope ratios across shell sections for $\delta^{18}\text{O}$ (A) and $\delta^{13}\text{C}$ (B), and differences in isotopic ratios among individual mussels and sites (C). Samples are numbered from the umbo to the ventral margin. Each series, connected by dashed lines, represents an individual mussel. In C each point represents the average values across samples for an individual mussel, with standard error bars.

Table 3: Pearson's correlations between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ for individual mussels. Significant correlations are in bold.

Site	Shell ID	t	df	p	Pearson's r
MF1 (ancient)	A	2.12	17	0.049	0.457
	B	3.67	16	0.002	0.676
	C	5.05	16	<0.001	0.784
MF2 (ancient)	D	7.02	10	<0.001	0.912
	E	4.23	18	0.001	0.706
	F	1.77	16	0.096	0.404
KD (modern)	G	-0.14	22	0.892	-0.029
	H	1.88	16	0.079	0.424
	I	-2.62	18	0.017	-0.526

4. DISCUSSION

By comparing freshwater mussel shells from both ancient and modern communities of the River Nene, we show that shells can provide a record of ecological change at the individual, population and community level. We show that ancient mussels (late 2nd to early 1st millennium BC) in the River Nene grew to a smaller size than modern mussels from analogous sites. These ancient mussels also

had lower year-to-year variability in growth than modern mussels. Stable isotope analysis showed $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ both varied among sites. $\delta^{13}\text{C}$ but not $\delta^{18}\text{O}$ was lower for modern compared to ancient mussels. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were positively correlated for ancient but not modern mussels. The shifts we found in mussel growth rate, as well as the loss of rare species, are likely to have occurred in response to anthropogenic environmental alteration, including nutrient and flow alteration.

4.1 Community composition

Mussel species composition was similar across ancient and modern populations between the two periods. However, the presence of fragmented *Pseudanodonta complanata* in ancient but not modern samples may reflect the regional decline of this species, which is listed as Near Threatened by Red Lists for both Europe (Cuttelod et al., 2011) and Great Britain (Seddon et al., 2014), and is a Priority Species under the UK Biodiversity Action Plan (BRIG, 2007). *Pseudanodonta complanata* is particularly sensitive to environmental disturbance (Ćmiel et al., 2019) and recent local declines or extirpations have been reported elsewhere in south-east England (Ollard & Aldridge, 2023) as well as elsewhere in Europe (Lewandowski & Kolodziejczyk, 2014; Ćmiel et al., 2019; Ożgo et al., 2020). The apparent absence of *P. complanata* from modern sites may therefore reflect the much more intensive anthropogenic disturbance of the modern Nene ecosystem, as well as the channelisation of the river leading to microhabitat homogenisation. This reflects broad patterns in the literature of reduced biodiversity and absence of ecologically sensitive species in modern compared to ancient mussel assemblages (Randklev et al., 2010; Wolverton et al., 2010; Mitchell & Peacock, 2014; Peacock et al., 2016; Mitchell, 2018; Popejoy et al., 2018; Mitchell & Childress, 2021; Mitchell et al., 2023).

4.2 Size and growth rate

Larger size in modern compared to ancient shells could have been caused by sampling bias or differential preservation. Since modern shells were sampled by hand rather than by sieving

sediments, this could have skewed modern samples towards larger, easier-to-find shells (Miller & Payne, 1988; Vaughn et al., 1997). It is also possible that larger ancient shells were less likely to be preserved, although Wolverton et al (2010) found no effect of shell size on preservation and we think it unlikely that this explains the differences we found.

Size and growth rate in mussels shows high plasticity within species, and can vary both between and within populations (Haag & Rypel, 2011; Butitta et al., 2021). Peacock and Seltzer (2008) state that “it has been noted many times that prehistoric mussel shell is smaller than modern shells from the same waterways”, attributing this to effects of land clearance, including changes in flow, increased nutrient availability, and altered substrate composition.

In our study, differences in flow regime between modern and ancient sites probably contributed to size and growth differences. The paleochannel tributary was shallow and slow-flowing, with possible periods of drying, while both modern sites are deeper with continuous year-round flow. Growth can be both negatively and positively associated with flow rate (Haag & Rypel, 2011): at low flows a positive flow-growth rate relationship is expected since increasing flow increases food availability (Schöne et al., 2007), while the energy expenditure of surviving in high flows may reverse this relationship (Rypel et al., 2008; Strayer, 2008). The very slow-flowing, sometimes nearly still water in the paleochannel is likely to have limited food delivery to mussels, while the modern sites rarely experience periods of extremely high flow. Similar effects of changing flow have been observed in ancient mussel assemblages elsewhere. Klippel et al (1978) attributed smaller size in prehistoric *Amblema plicata* in the Pomme de Terre River, Mississippi, to slower flow at the ancient site. Many shell middens from North America in which ancient shells are smaller than modern equivalents approximately coincided with the Holocene Climatic Optimum, which likely caused drier conditions that led to decreased stream size, depth and flow (Peacock, 2000). In contrast, larger mussel size has also been reported for mussels in lentic compared with adjacent lotic habitats, with *U. pictorum* in marinas in the River Thames growing larger than in the adjacent river, attributed to higher

480 temperatures and greater algal growth (Zieritz & Aldridge, 2009). Variation in flow can also affect
481 mussel growth. DuBose et al. (2022) found a positive relationship between mussel growth and flow
482 stability, with greater flow stability associated with larger mussel size. In our study, although the
483 modern sites do not undergo periods of drying as the paleochannel may have done, flow is still
484 relatively variable and the variability in interannual growth might also reflect this.

485 Nutrient status is also likely to have affected growth rates. The paleochannel probably had a lower
486 trophic state than the modern Nene, which experiences significant anthropogenic nutrient
487 enrichment and is classified as eutrophic (Rose & Balbi, 1997). Analysis of multiple aquatic floral and
488 invertebrate assemblages from the paleochannel suggest that water was very slow flowing, clear and
489 well-oxygenated (Ballantyne, 2024; Cameron, 2024; Langdon, 2024; Timberlake, 2024; Vickers et al.,
490 2024), with abundant macrophytes. This is supported by the presence in ancient samples of
491 *Pseudanodonta complanata* and its apparent absence from modern sites, since this species appears
492 more sensitive to eutrophication than other European unionid mussels (Lewandowski &
493 Kolodziejczyk, 2014).

494 Nutrient enrichment can lead to increased food availability for filter-feeding mussels, resulting in
495 larger size (Arter, 1989; Dunca et al., 2005; Versteegh et al., 2010). In North America, eutrophication
496 was associated with mussels in the Illinois river increasing in size steadily across samples from 1894
497 onwards, with the largest sizes being observed in most recent samples (Fritts et al., 2017), while
498 shells from archaeological assemblages dated to c.850 had smaller average size, with individuals
499 collected in 2013 over 50% larger than conspecifics from 850. In the Thames, *U. pictorum* was found
500 to have smaller average size in 2020 compared to samples from 1964, possibly due to decreases in
501 nutrient enrichment (Ollard & Aldridge, 2023). Conversely, very eutrophic ecosystems can result in
502 higher mortality and earlier maturation at smaller size (Müller et al., 2021).

503 Population age structure can also alter average size of mussels – in the Sunflower River, Mississippi,
504 archaeological samples contained large numbers of small valves, indicating healthy recruitment,

while no recruitment was evident for several species in modern samples (Mitchell & Peacock, 2014). It is therefore possible that the presence of larger mussels in the modern populations in our results is due to a geriatric population with low or no reproduction. This possible lack of recruitment could be caused by, among other factors, the loss of fish that are needed as hosts by mussel larvae to allow metamorphosis (Modesto et al., 2018). Although age distribution did differ among our study populations, the fact that differences in age did not coincide with differences in length (for example, Must Farm 2 had the highest average age but second-lowest average length) suggests that differences in length are unlikely to be an effect of differences in age structure.

Temperature is generally positively correlated with growth rate (Schöne et al., 2004). A study of *Unio tumidus* from modern populations and the Early Middle Ages from the Oder Estuary, Poland, found that the milder temperatures of the Medieval Climatic Optimum may have caused ancient mussels to reach both older age and larger size than modern individuals (Czerniejewski et al., 2021). While shallower depth in the paleochannel may have resulted in higher summer temperatures, the lack of clear differences in $\delta^{18}\text{O}$ suggests temperature is unlikely to have been a key driver. This observation is further supported by the ostracod populations in the same palaeochannel deposits as our shell samples, which indicate similar water temperatures to the present day (Timberlake, 2024: 72–73).

Finally, fouling by *Dreissena polymorpha* can also lead to poorer condition in unionids (Sousa et al., 2011) and possibly reduced growth (Dzierżyńska-Białończyk et al., 2018), but we did not find this effect: despite the arrival of *Dreissena*, unionids now reach larger sizes.

Size and growth also differed between the two modern sites. Compared to the main channel site, King's Dyke had significantly lower alkalinity over the period of mussels' growth. Higher alkalinity has been suggested to increase shell thickness, although evidence is mixed (Strayer et al., 2021). The main channel Nene site is also wider and deeper, with higher flow. It had slightly higher average values for nutrient (nitrate and phosphate) concentrations, although these differences were not statistically significant when a multiple-comparison correction was applied.

We are not aware of other studies that have reported a change in year-to-year variability in mussel growth rates. The increase in variability we found in modern compared to ancient mussels may be associated with the higher flow at both modern sites compared to the Must Farm paleochannel, which could be linked to greater flow variability and differences in peak flow such as flood events. However, the paleochannel was apparently also subject to flow variability and episodic drying (French, 2024), and it might be expected that mussels in a deeper channel with higher flow should experience more buffering against variability of this type. Alternatively, this increased variability could be linked to greater climate variability resulting from anthropogenic climate change (IPCC, 2014). This could for example result in more variability in growing-season temperature between years, or a longer period of the year during which growth occurs. Increasing variability in year-to-year growth may be a signal of increasing interannual differences in environmental stress or favourability, and hence mussel condition, more broadly. This could have wider population-level implications, for example for recruitment. However, further investigation is needed to elucidate the potential significance of this pattern, and to identify whether it is a more broadly occurring trend.

4.3 Isotopic composition

4.3.1 $\delta^{18}\text{O}$

Bivalve shell $\delta^{18}\text{O}$ provides information largely on temperature at the time of shell formation (Schöne et al., 2004; Kukolich & Dettman, 2021), as well as habitat source water (Versteegh et al., 2009) and the balance of evaporation and precipitation. Our sampling resolution of c. 2 mm intervals was not sufficiently fine to detect a clear signal of $\delta^{18}\text{O}$ seasonality. Although there was some differentiation in mussel shell $\delta^{18}\text{O}$ between sites, potentially reflecting differences in temperature or flow regime (Lewis et al., 2017; Apolinarska & Kurzawska, 2020; Kukolich & Dettman, 2021), there was no clear difference between time periods. We expected $\delta^{18}\text{O}$ to be higher for the ancient samples, since these came from a channel that was probably slower-flowing and shallower than modern samples. However, we found no such differences. This suggests that the differences in growth rates and

variability we found were not due to climatic or temperature differences, increasing confidence that eutrophication may instead be the driver of growth rate change. Other comparisons of modern and ancient shell $\delta^{18}\text{O}$ have similarly found no differences between modern and ancient shell $\delta^{18}\text{O}$ (Peacock & Seltzer, 2008; Çakırlar & Şeşen, 2013; Vonhof et al., 2013; Apolinarska & Kurzawska, 2020).

4.3.2 $\delta^{13}\text{C}$

We found differences in shell $\delta^{13}\text{C}$ between but not within time periods, with both Must Farm sample groups showing enriched $\delta^{13}\text{C}$ compared to the modern King's Dyke mussels. This fits the hypothesis that the paleochannel was shallower and slow-flowing with abundant macrophytes, since higher rates of photosynthesis and preferential sequestration and removal from the DIC pool of ^{12}C by plants would increase DIC $\delta^{13}\text{C}$ (Kelemen et al., 2017; Apolinarska & Kurzawska, 2020). While we might also expect modern nutrient enrichment also to increase photosynthesis and result in enriched $\delta^{13}\text{C}$, the greater shallowness and slower flow of the paleochannel means DIC was likely to have been more strongly influenced by rates of photosynthesis than in the modern King's Dyke. In contrast to these results, Fritts et al (2017) found an increase in shell $\delta^{13}\text{C}$ between 1894 and 1966, although there was a subsequent decrease to 2013. This accompanied an increase in growth rate like the one we report. The authors suggest that the increase in $\delta^{13}\text{C}$ resulted from sewage pollution, which increased during the first half of the 20th century before starting to decrease during the second half; size and growth rate increases are also attributed to anthropogenic nutrient enrichment. While we found similar effects for size and growth, we found the opposite effect for $\delta^{13}\text{C}$, which decreased instead of increasing for modern samples. It is possible that this is due to differences in metabolic carbon sources and contribution to shell $\delta^{13}\text{C}$, for example if more recent samples from the Illinois River reflect an increased dietary contribution of heterotrophs compared to autotrophs. However, the absolute values for $\delta^{13}\text{C}$ for the Illinois River mussels are significantly lower than for our study (between -29‰ and -25‰), suggesting that controls on DIC and shell carbon are very different from

the Nene, and may not be directly comparable. Also, the absence from the Illinois study of baseline values prior to anthropogenic alteration means it is difficult to compare in context with our study.

It is also possible that the approximately 2‰ decrease in $\delta^{13}\text{C}$ for modern compared to ancient mussels results from the Suess effect (Keeling, 1979; Rubino et al., 2013). However, other studies have not reported this difference in $\delta^{13}\text{C}$ between ancient and modern mussels (Peacock & Seltzer, 2008; Bar-Yosef Mayer et al., 2012; Çakırlar & Şeşen, 2013; Lewis et al., 2017; Van Plantinga & Grossman, 2018; Apolinarska & Kurzawska, 2020). Unlike us, Fritts et al (2017) found increases in shell $\delta^{13}\text{C}$ between 1894 and 1966, but a subsequent decrease to 2013. This accompanied an increase in growth rate like the one we report. The authors suggest that the increase in $\delta^{13}\text{C}$ resulted from sewage pollution, which increased during the first half of the 20th century before starting to decrease during the second half; size and growth rate increases are also attributed to anthropogenic nutrient enrichment. Although nutrient enrichment has almost certainly occurred in the River Nene, with similar effects on size and growth, we found the opposite effect on $\delta^{13}\text{C}$.

The positive correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in ancient but not modern samples, indicating in-phase variation, fits with other evidence for a more hydrologically closed system at the ancient site. In lakes where residence time is longer and evaporation is a key control on isotopic composition, strong positive correlation is expected, while in river systems there may be no or a negative correlation (Schöll-Barna et al., 2012; Vonhof et al., 2013; Buskirk et al., 2016). The lack of correlation in modern samples might also reflect a greater or more variable contribution of metabolic carbon to shell composition; in any case the unknown contribution of metabolic carbon to shell composition in our data means it should be treated with caution.

5. Conclusions

We found that ancient *Unio pictorum* mussels had lower size and growth, and lower variability in year-to-year growth, than analogous modern mussels. In addition, the ancient mussels had enriched $\delta^{13}\text{C}$, but not $\delta^{18}\text{O}$, compared to modern mussels. These differences probably stem largely from local

environmental changes, in particular modern day increases in channel depth and flow rate and greater food availability resulting from anthropogenic nutrient enrichment. Climatic effects and temperature do not appear to have played a significant role in changing mussel size and growth patterns, as indicated by the lack of difference in $\delta^{18}\text{O}$. In addition, the sensitive, threatened *Pseudanodonta complanata* appears to have been lost from mussel communities, possibly linked to nutrient enrichment and eutrophication. This reflects a broad global trend of loss of disturbance-sensitive mussel species from ecosystems as well as a general increase in mussel size and growth rate among modern individuals; while the drivers of this are likely to be site-specific, the effects of anthropogenic habitat alteration, land use change and particularly resulting nutrient enrichment are common themes. Given the recent rise in interest in fenland restoration, including peatland rewetting, these conclusions reflect the importance of using having a long-term perspective on changes to aquatic habitats and water quality, and the communities they support, using data on historical ecosystems to inform present-day conservation and restoration and to avoid shifting baseline syndrome. Our results demonstrate the utility of studying ancient mussel community assemblages to act as baselines for reconstructing communities prior to significant human disturbance, as well as to inform about past environmental conditions.

DATA AVAILABILITY

Data are available through the Apollo Digital Repository at <https://doi.org/10.17863/CAM.108461> (Ollard et al., 2024).

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AUTHOR CONTRIBUTIONS

Isobel Ollard: Conceptualisation, Methodology, Formal analysis, Investigation, Writing – Original Draft, Writing – Review and Editing, Visualisation, Project administration. **Rachel Ballantyne:** Conceptualisation, Methodology, Resources, Writing – Review and Editing, Supervision, Project administration. **David Aldridge:** Conceptualisation, Methodology, Investigation, Writing – Review and Editing, Supervision.

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