*Global Change Biology*

WILEY

Global Change Biology

RESEARCH ARTICLE OPEN ACCESS

# Freshwater Mussel (*Unio pictorum*) Shells Reveal Hydrological and Environmental Change From 1300 BC to the Present Day

Isobel Ollard[1] | Rachel Ballantyne[2,3] | David C. Aldridge[1]

[1]Department of Zoology, David Attenborough Building, University of Cambridge, Cambridge, UK | [2]Department of Archaeology, University of Cambridge, Cambridge, UK | [3]School of Archaeology, University of Oxford, Oxford, UK

**Correspondence:** Isobel Ollard (isobelollard@gmail.com)

**Received:** 30 May 2024 | **Revised:** 10 September 2024 | **Accepted:** 14 September 2024

**Keywords:** baseline | flow | growth rate | nutrient enrichment | palaeoecology | stable isotope

## 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 palaeochannel 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 ($\delta^{18}O$ and $\delta^{13}C$) composition were compared between the populations. Size and the von Bertalanffy growth parameter $L_\infty$ differed among all four populations. Mean lengths and $L_\infty$ were higher in the two modern populations (mean lengths 77.3 ± SE 0.8 and 73.8 ± SE 1.1 mm, $L_\infty$ 91.8 ± 5.4 and 79.0 ± 8.1 mm) than the ancient populations (mean lengths 58.1 ± SE 1.6 mm and 68.4 ± SE 0.9 mm; $L_\infty$ 71.5 ± 16.9 and 76.8 ± 6.2 mm). Modern individuals also showed greater variation in age-corrected year-to-year growth. $\delta^{13}C$ 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. $\delta^{18}O$ and $\delta^{13}C$ 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.

## 1 | Introduction

As the global degradation of ecosystems progresses as a result of long-acting anthropogenic ecosystem alteration, constructing an accurate representation of intact, nondegraded 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, Sousa, and Mesquita-Joanes 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 and Downing 2001). Due to this long history of degradation in mussel communities, it can be difficult to know what intact, predisturbance mussel communities may have looked like (e.g., 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; Wolverton, Randklev, and Kennedy 2010; Pickard, Boroneant, and Bonsall 2017). Studying these shells can provide valuable information about mussel communities in the preIndustrial 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, Randklev, and Kennedy 2010) and the possibility that shell deposits may have been assembled over long timescales (Nielsen and Helama 2021).

Species identity can contribute to environmental reconstruction, since the presence of species with particular tolerances can provide information on environmental conditions (Pickard, Boroneant, and Bonsall 2017) such as water clarity or turbidity and silt levels (e.g., Mitchell and Peacock 2014; Mitchell, Childress, and French 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, Randklev, and Kennedy 2010; Peacock, Mitchell, and Jenkins 2016; Mitchell 2018; Mitchell, Childress, and French 2023), reflecting disturbance, degradation and environmental homogenisation across ecosystems.

Patterns of mussel growth can also provide information about past environments and populations (Pickard, Boroneant, and Bonsall 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, Reische, and Lohmann 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, Haag, and Findlay 2008; Versteegh et al. 2009). Mussel size and growth rates are highly variable both within and between populations (Haag and Rypel 2011; DuBose et al. 2022) and are sensitive to a range of environmental factors, including temperature (Schöne et al. 2004; Lundquist, Worthington, and Aldridge 2019), food availability (Arter 1989; Versteegh et al. 2010; Müller et al. 2021) and flow regime (Rypel, Haag, and Findlay 2008; Haag and Rypel 2011; Butitta, Rypel, and Stanley 2021). Biotic factors including competition, parasites, disease and predation can also influence variability (Meira, Byers, and Sousa 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 $\delta^{18}O$ and $\delta^{13}C$ isotopes. Bivalve shell carbonate is precipitated in oxygen-isotopic equilibrium with ambient water (Dettman, Reische, and Lohmann 1999), and shell $\delta^{18}O$ is dependent on both $\delta^{18}O$ of ambient water and temperature during shell formation. Ambient water $\delta^{18}O$ depends on the balance of evaporation and precipitation, with $\delta^{18}O$ enriched in bivalve shells formed during warm, dry periods (Lewis et al. 2017). Elevated $\delta^{18}O$ has been reported for modern compared to ancient shells (van Plantinga and 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 $\delta^{18}O$ in ancient shells, possibly reflecting intense summer evaporation at the time of shell formation. Shell $\delta^{18}O$ can provide an accurate record of temperature (Kukolich and Dettman 2021), so incremental samples from freshwater mussel shells generally show clear seasonality in $\delta^{18}O$ (Pfister et al. 2018). In addition, higher $\delta^{18}O$ is expected in mussels from lakes and shallow, low-flow environments compared to flowing rivers (Vonhof et al. 2013; Apolinarska and Kurzawska 2020), since longer residence time allows greater evaporation of $^{16}O$.

$\delta^{13}C$ in freshwater mussel shell is largely dependent on the isotopic composition of ambient dissolved inorganic carbon (DIC). DIC $\delta^{13}C$ can vary with factors including precipitation (Schöll-Barna et al. 2012), water depth and residence time (Apolinarska and Kurzawska 2020), lake versus river habitat (Kaandorp, Wesselingh, and Vonhof 2006; Vonhof et al. 2013), reproduction (Dettman, Reische, and Lohmann 1999), salinity (McConnaughey and Gillikin 2008) and catchment vegetation (Kelemen et al. 2017). A generally smaller but variable contribution to shell $\delta^{13}C$ derives from metabolic carbon (Dettman, Reische, and Lohmann 1999; McConnaughey and Gillikin 2008; Lewis et al. 2017), which decreases shell $\delta^{13}C$. This variable contribution can obscure environmental effects on shell $\delta^{13}C$ (Dettman, Reische, and Lohmann 1999; Kelemen et al. 2017). In addition, the combustion of fossil fuels in the postindustrial era has led to an overall depletion of atmospheric $\delta^{13}C$ 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 $\delta^{13}C$ is complex and poorly understood (Apolinarska and Kurzawska 2020).

Stable isotope analysis has been used to examine changing climate (Peacock and Seltzer 2008; Bar-Yosef Mayer et al. 2012; Schöll-Barna et al. 2012), habitat conditions (Vonhof et al. 2013; van Plantinga and 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 postshell 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 postdating 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 $\delta^{18}O$ and $\delta^{13}C$ 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; Figure 1). The site is located on a palaeochannel 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 to late 16th centuries BC to the first century AD (Ballantyne, Knight, and Robinson Zeki 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 postdating 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 4 mm 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 *P. 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 (Figure 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 received 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, Worthington, and Aldridge 2019).

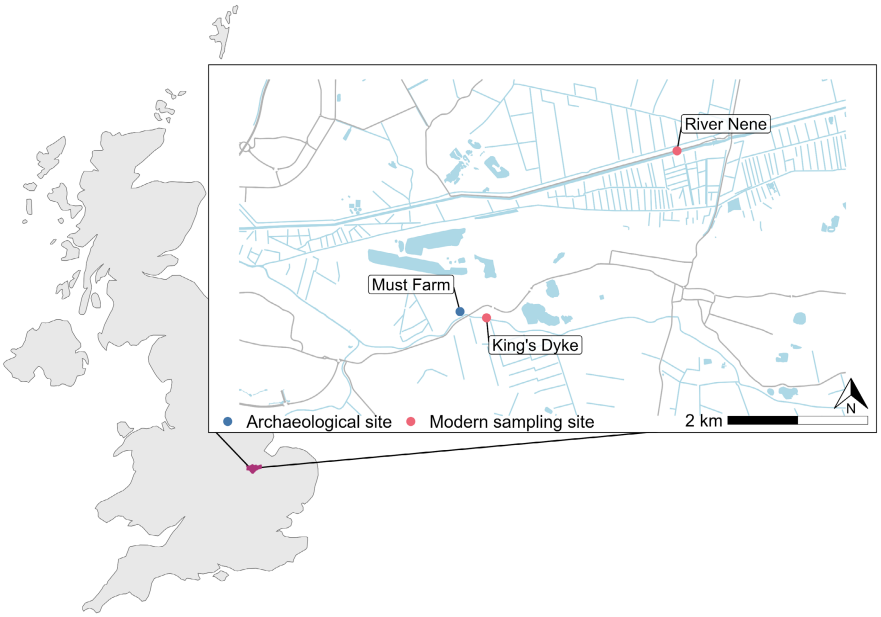


**FIGURE 1** | Map of sampling sites. The Peterborough region where sampling took place is marked on the larger map. Map created using data from OpenStreetMap.

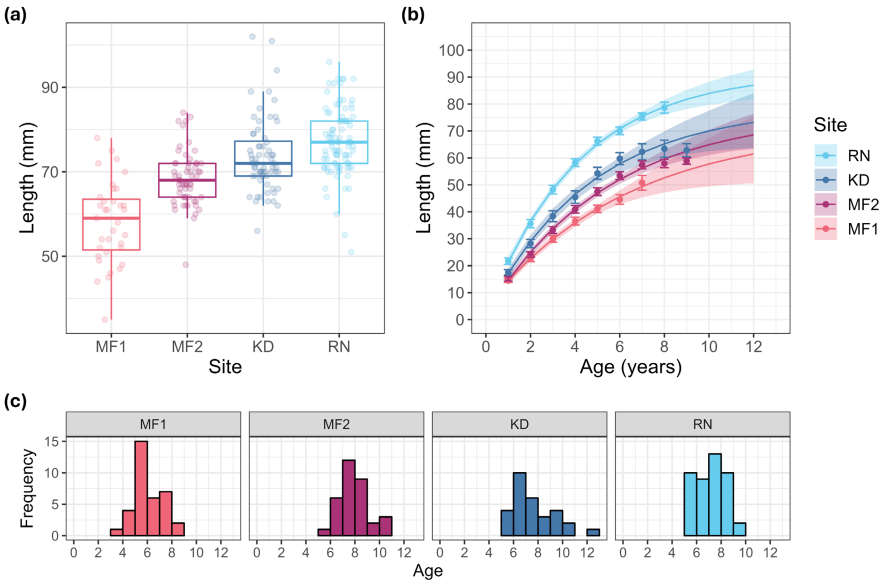


**FIGURE 2** | Length (a), fitted von Bertalanffy growth curves (b) and age-frequency plots (c) for each site. In (a), the boxes show median and interquartile range, and the endpoints of the whiskers extend to 1.5 × IQR beyond the boxes. In (b), growth curves are shown with bootstrapped 95% confidence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

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, Taylor, and Eberhard 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, Randklev, and Kennedy 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 at a Euclidean distance of 3.25 km 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 at a Euclidean distance of 7.5 km 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 sometime 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 one to two 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 palaeochannel tributary was shallow and slow-flowing (Ballantyne, Knight, and Robinson Zeki 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 (2–3 m) 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 presettlement 'Must Farm 1' had TP of 72–74 $\mu g\,L^{-1}$ while the postsettlement 'Must Farm 2' had TP of 35–65 $\mu g\,L^{-1}$. 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.

## 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 $\delta^{18}O$ and $\delta^{13}C$ 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 palaeochannel could lead to large site-based differences in $\delta^{18}O$ and $\delta^{13}C$ 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 $70 \pm 10$ 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, 820–890, 1070–1095, 1200–1600 and 1760–1820 $cm^{-1}$ were used in the analysis, using the methodology described by Loftus, Rogers, and Lee-Thorp (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 and 2 samples and the King's Dyke samples were then sent for isotope analysis for $\delta^{13}C$ and

**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 ± SE) | River Nene (mean ± SE) |
|---|---|---|---|---|
| pH | 8 | 8 | n.d. | n.d. |
| Alkalinity ($mg\,L^{-1}$ $CaCO_3$) | 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 |

$\delta^{18}O$. 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 h at 70°C 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 two 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 $\delta^{13}C$ and ±0.10 ppm for $\delta^{18}O$.

All collected data are publicly available (Ollard, Ballantyne, and Aldridge 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; (Equation 1); von Bertalanffy 1938), which has been shown to represent bivalve growth patterns (Hastie, Young, and Boon 2000). This model includes three constants: $L_\infty$ is the 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. Ninety-five per cent 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 ($L_\infty$, $K$ and $t_0$) varied among populations, and a set of further models in which each coefficient, in turn, was constrained, as well as a model where no coefficients varied among populations (Equations 2–6). We compared each constrained model against the general model using likelihood-ratio chi-squared 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-squared test $p$-values.

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

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

$$L_t = L_{\infty(\text{site})}\left(1 - e^{-K_{(\text{site})}(t-t_{0(\text{site})})}\right) \tag{2}$$

$H_0$: All parameters vary between populations.

$$L_t = L_\infty\left(1 - e^{-K_{(\text{site})}(t-t_{0(\text{site})})}\right) \tag{3}$$

$H_1$: $L_\infty$ constant across populations.

$$L_t = L_{\infty(\text{site})}\left(1 - e^{-K(t-t_{0(\text{site})})}\right) \tag{4}$$

$H_2$: $K$ constant across populations.

$$L_t = L_{\infty(\text{site})}\left(1 - e^{-K_{(\text{site})}(t-t_0)}\right) \tag{5}$$

$H_3$: $t_0$ constant across populations.

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right) \tag{6}$$

$H_4$: All parameters constant across populations.

We also analysed within-mussel interannual growth variability in the length of annual growth increments (rather than lengths, to remove year-to-year nonindependence). We fitted a negative exponential with a constant term (Equation 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 Equation (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 ($\sigma$ GI) values for each individual mussel to give a measure of within-individual interannual growth variability. A high $\sigma$ 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 $\sigma$ 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. $\sigma$ GI values were log-transformed for subsequent analyses to meet assumptions of normality and homoscedasticity. We compared the effect of site and time (modern vs. ancient) on log-transformed $\sigma$ GI using two-way ANOVA.

$$I_t = ae^{-bt} + c \tag{7}$$

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.

$$\text{GI}_t = I_t / E_t \tag{8}$$

Method for calculating growth index values where $\text{GI}_t$ = 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 Equation (7).

Isotope data were analysed using linear mixed models, using the packages *lme4* (Bates et al. 2015) for model construction, *lmtest* (Zeileis and Hothorn 2002) for model testing and *emmeans* (Lenth 2024) for pairwise comparisons. Models were constructed with each isotope ($\delta^{18}O$ and $\delta^{13}C$) 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 intersample 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 $\delta^{18}O$ (Kelemen et al. 2017) and $\delta^{13}C$ (Yoshimura et al. 2015; Kukolich and Dettman 2021). Other studies have reported declining $\delta^{13}C$ with age (McConnaughey and Gillikin 2008; Błażejowski et al. 2013; Kelemen et al. 2017), although Kaandorp, Wesselingh, and Vonhof (2006) found the opposite pattern. Błażejowski et al. (2013) also reported a relationship between $\delta^{18}O$ and age. We tested for ontogenetic effects of age–isotope correlation for both oxygen and carbon isotopes using 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 $\delta^{18}O$ with age, and a further one (shell 17 from KD; Pearson's coefficient = −0.485) showed a significant decrease in $\delta^{13}C$ 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 $R^2 = 0.375$; Figure 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 ± SE 0.8 mm), followed by King's Dyke (73.8 ± SE 1.1 mm), Must Farm 2 (68.4 ± SE 0.9 mm) and Must Farm 1 (58.1 ± SE 1.6 mm).

Growth curves also differed among all sites (models $H_0$ vs. $H_4$; $X^2_{(9)} = 565.93$, $p < 0.001$; Figure 2b). The VBGF coefficient $L_\infty$ differed significantly between sites (models $H_0$ vs. $H_1$; $X^2_{(3)} = 12.69$, $p = 0.005$). There were no significant differences between sites in the parameters $K$ (models $H_0$ vs. $H_2$; $X^2_{(3)} = 4.97$, $p = 0.174$) or $t_0$ (models $H_0$ vs. $H_3$; $X^2_{(3)} = 3.11$, $p = 0.375$). 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 ($p < 0.001$; Figure 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 ± SE 0.21 years), followed by King's Dyke (7.13 ± SE 0.32 years), River Nene (6.66 ± SE 0.18 years) and Must Farm 1 (5.57 ± SE 0.20 years).

### 3.2 | Interannual Growth Variability

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

### 3.3 | $\delta^{13}C$ and $\delta^{18}O$ Isotope Analysis

For $\delta^{18}O$, 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 ($X^2_{(2)} = 7.18$, $p = 0.028$; Figure 4). There were no significant pairwise differences among sites ($p > 0.05$ for all pairwise comparisons). Similarly, for $\delta^{13}C$, site was also a significant predictor of isotopic ratio in mixed models that included individual mussel as a random effect ($X^2_{(2)} = 21.02$, $p < 0.001$). 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).

**TABLE 2** | Von Bertalanffy growth parameters for each population.

| Site | $L_\infty$ | $K$ | $t_0$ | $RSE_{(df)}$ |
|---|---|---|---|---|
| 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 | $5.52_{(3,239)}$ |

Abbreviations: $K$, rate at which asymptotic length is attained; $L_\infty$, asymptotic length; RSE, residual standard error; $t_0$, age at which length equals zero.

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

$\delta^{18}O$ and $\delta^{13}C$ 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 $\delta^{18}O$ and $\delta^{13}C$ 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 $\delta^{18}O$ and $\delta^{13}C$, 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.

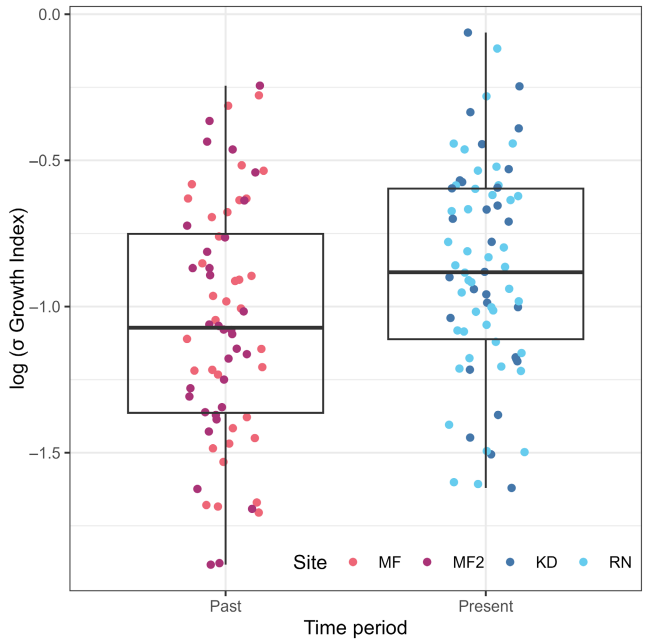


**FIGURE 3** | Within-mussel interannual variability in growth, measured as log (σ growth index).

## 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 second to early first 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 $\delta^{13}C$ and $\delta^{18}O$ both varied among sites. $\delta^{13}C$ but not $\delta^{18}O$ was lower for modern compared to ancient mussels. $\delta^{13}C$ and $\delta^{18}O$ 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 *P. 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, Seddon, and Neubert 2011) and Great Britain (Seddon, Killeen, and Fowles 2014), and is a Priority Species under the UK Biodiversity Action Plan (BRIG 2007). *P. 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 and Aldridge 2023) as well as elsewhere in Europe (Lewandowski and 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, Randklev, and Kennedy 2010; Mitchell and Peacock 2014; Peacock, Mitchell,

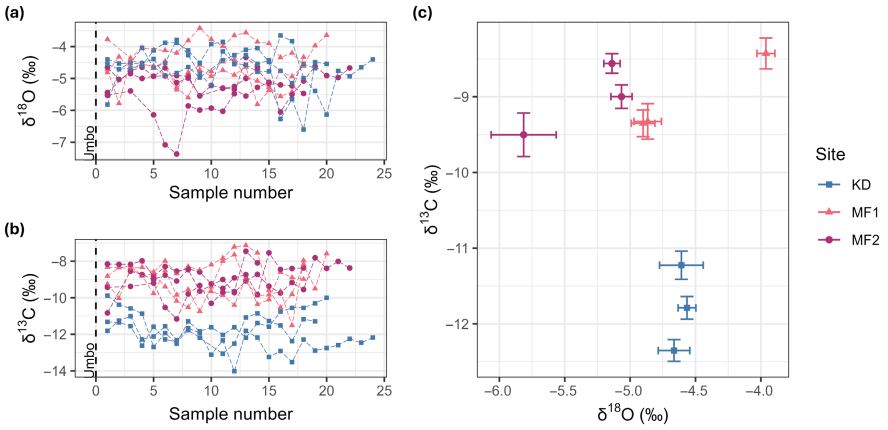


**FIGURE 4** | Isotope ratios across shell sections for $\delta^{18}O$ (a) and $\delta^{13}C$ (b), and differences in isotopic ratios among individual mussels and sites (c). Samples are numbered from the umbo to the ventral margin. Each series, connected by dashed lines, represents an individual mussel.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

**TABLE 3** | Pearson's correlations between $\delta^{18}O$ and $\delta^{13}C$ for individual mussels.

| 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** |

*Note:* Significant correlations are in bold.

and Jenkins 2016; Mitchell 2018; Popejoy et al. 2018; Mitchell and Childress 2021; Mitchell, Childress, and French 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 and Payne 1988; Vaughn, Taylor, and Eberhard 1997). It is also possible that larger ancient shells were less likely to be preserved, although Wolverton, Randklev, and Kennedy (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 show high plasticity within species, and can vary both between and within populations (Haag and Rypel 2011; Butitta, Rypel, and Stanley 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 the 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 palaeochannel 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 and Rypel 2011): at low flows, a positive flow–growth rate relationship is expected since increasing flow increases food availability (Schöne et al. 2007), while energy expenditure of surviving in high flows may reverse this relationship (Rypel, Haag, and Findlay 2008; Strayer 2008). The very slow-flowing, sometimes nearly still water in the palaeochannel 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, Celmer, and Purdue (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 temperatures and greater algal growth (Zieritz and Aldridge 2009). Variation in flow can also affect mussel growth. DuBose et al. (2022) found a positive relationship between mussel growth and flow stability, with greater flow stability associated with larger mussel size. In our study, although the modern sites do not undergo periods of drying as the palaeochannel may have done, flow is still relatively variable and the variability in interannual growth might also reflect this.

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

Nutrient enrichment can lead to increased food availability for filter-feeding mussels, resulting in larger size (Arter 1989; Dunca, Schöne, and Mutvei 2005; Versteegh et al. 2010). In North America, eutrophication was associated with mussels in the Illinois River increasing in size steadily across samples from 1894 onwards, with the largest sizes being observed in most recent samples (Fritts et al. 2017), while shells from

archaeological assemblages dated to c. 850 had smaller average size, with individuals collected in 2013 over 50% larger than conspecifics from 850. In the Thames, *U. pictorum* was found to have smaller average size in 2020 compared to samples from 1964, possibly due to decreases in nutrient enrichment (Ollard and Aldridge 2023). Conversely, very eutrophic ecosystems can result in higher mortality and earlier maturation at smaller size (Müller et al. 2021).

Population age structure can also alter average size of mussels—in the Sunflower River, Mississippi, archaeological samples contained large numbers of small valves, indicating healthy recruitment, while no recruitment was evident for several species in modern samples (Mitchell and 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 (e.g., 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 *U. 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 palaeochannel may have resulted in higher summer temperatures, the lack of clear differences in $\delta^{18}O$ suggests that 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 *D. polymorpha* can also lead to poorer condition in unionids (Sousa, Pilotto, and Aldridge 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, Hamilton, and Malcom 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 palaeochannel, which could be linked to greater flow variability and differences in peak flow such as flood events. However, the palaeochannel 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 | $\delta^{18}O$

Bivalve shell $\delta^{18}O$ provides information largely on temperature at the time of shell formation (Schöne et al. 2004; Kukolich and 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 $\delta^{18}O$ seasonality. Although there was some differentiation in mussel shell $\delta^{18}O$ between sites, potentially reflecting differences in temperature or flow regime (Lewis et al. 2017; Apolinarska and Kurzawska 2020; Kukolich and Dettman 2021), there was no clear difference between time periods. We expected $\delta^{18}O$ 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 $\delta^{18}O$ have similarly found no differences between modern and ancient shell $\delta^{18}O$ (Peacock and Seltzer 2008; Çakirlar and Şeşen 2013; Vonhof et al. 2013; Apolinarska and Kurzawska 2020).

### 4.3.2 | $\delta^{13}C$

We found differences in shell $\delta^{13}C$ between but not within time periods, with both Must Farm sample groups showing enriched $\delta^{13}C$ compared to the modern King's Dyke mussels. This fits the hypothesis that the palaeochannel 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 $\delta^{13}C$ (Kelemen et al. 2017; Apolinarska and Kurzawska 2020). While we might also expect modern nutrient enrichment also to increase photosynthesis and result in enriched $\delta^{13}C$, the greater shallowness and slower flow of the palaeochannel mean that 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$, 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 $\delta^{13}C$, 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 $\delta^{13}C$ 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 that it is difficult to compare in context with our study.

It is also possible that the approximately 2‰ decrease in $\delta^{13}C$ 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 $\delta^{13}C$ between ancient and modern mussels (Peacock and Seltzer 2008; Bar-Yosef Mayer et al. 2012; Çakirlar and Şeşen 2013; Lewis et al. 2017; van Plantinga and Grossman 2018; Apolinarska and Kurzawska 2020). Unlike us, Fritts et al. (2017) found increases in shell $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$.

The positive correlation between $\delta^{18}O$ and $\delta^{13}C$ 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 that it should be treated with caution.

## 5 | Conclusions

We found that ancient *U. 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 $\delta^{13}C$, but not $\delta^{18}O$, 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 $\delta^{18}O$. In addition, the sensitive, threatened *P. 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 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.

### Author Contributions

**Isobel Ollard:** conceptualization, formal analysis, investigation, methodology, project administration, visualization, writing – original draft, writing – review and editing. **Rachel Ballantyne:** conceptualization, methodology, project administration, resources, supervision, writing – review and editing. **David C. Aldridge:** conceptualization, investigation, methodology, supervision, writing – review and editing.


### Acknowledgements

We thank Maggie Chen, Mark Knight and Mike Allen for assistance with fieldwork and sampling, Emma Loftus for discussions on FTIR and detecting diagenetic alteration, Tonko Rajkovača for assistance with shell sectioning, Simon Crowhurst for assistance with shell drilling, Caterina Zaggia for assistance with FTIR and Josh Brian for discussions on data analysis. Stable isotope Gasbench analysis was performed by James Rolfe at the Godwin Laboratory, Department of Earth Sciences, University of Cambridge. Initial concepts for collaboration with colleagues at the Department of Archaeology, University of Cambridge, were facilitated by Tamsin O'Connell. The Must Farm excavations were conducted by the Cambridge Archaeological Unit, University of Cambridge, and jointly funded by Historic England and Forterra, which included the employment of Rachel Ballantyne at the McDonald Institute for Archaeological Research, University of Cambridge. This analysis was conducted as an independent research project. Isobel Ollard was supported by a Whitten studentship. David Aldridge was supported by a Dawson Fellowship at St Catharine's College, Cambridge and by Corpus Christi College, Cambridge. We thank two anonymous reviewers for their comments, which significantly improved the manuscript.


### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

Data including processed environmental data are available through the Apollo Digital Repository at https://doi.org/10.17863/CAM.108461

(Ollard, Ballantyne, and Aldridge 2024). Original environmental data can be viewed at the UK Environment Agency Water Quality Monitoring Archive (https://environment.data.gov.uk/water-quality/view/).

## References


Allen, M. 2010. *Assessment of Molluscs Must Farm Pit Timber Alignments. An Archaeological and Environmental Evaluation. Part 2*, 22–27. Cambridge: Cambridge Archaeological Unit. https://doi.org/10.5284/1028094.

Anthony, J. L., and J. A. Downing. 2001. "Exploitation Trajectory of a Declining Fauna: A Century of Freshwater Mussel Fisheries in North America." *Canadian Journal of Fisheries and Aquatic Sciences* 58: 2071–2090. https://doi.org/10.1139/f01-130.

Apolinarska, K., and A. Kurzawska. 2020. "Can Stable Isotopes of Carbon and Oxygen Be Used to Determine the Origin of Freshwater Shells Used in Neolithic Ornaments From Central Europe?" *Archaeological and Anthropological Sciences* 12: 15. https://doi.org/10.1007/s12520-019-00978-2.

Arter, H. E. 1989. "Effect of Eutrophication on Species Composition and Growth of Freshwater Mussels (Mollusca, Unionidae) in Lake Hallwil (Aargau, Switzerland)." *Aquatic Sciences* 51: 87–99. https://doi.org/10.1007/BF00879296.

Balbi, D. M. 2000. "Suspended Chlorophyll in the River Nene, a Small Nutrient-Rich River in Eastern England: Long-Term and Spatial Trends." *Science of the Total Environment* 251–252: 401–421. https://doi.org/10.1016/S0048-9697(00)00419-8.

Ballantyne, R., M. Knight, and I. Robinson Zeki. 2024. "River." In *Must Farm Pile-Dwelling Settlement: Volume 1. Landscape, Architecture and Occupation*, edited by M. Knight, R. Ballantyne, M. Brudenell, A. Cooper, D. Gibson, and I. Robinson Zeki, 41–64. Cambridge: McDonald Institute for Archaeological Research. https://doi.org/10.17863/CAM.106697.

Ballantyne, R. 2024. "Seeds and Fruits." In *Must Farm Pile-Dwelling Settlement: Volume 2. Specialist Reports*, edited by R. Ballantyne, A. Cooper, D. Gibson, M. Knight, and I. Robinson Zeki, 57–64. Cambridge: McDonald Institute for Archaeological Research. https://doi.org/10.17863/CAM.106698.

Bar-Yosef Mayer, D. E., M. J. Leng, D. C. Aldridge, C. Arrowsmith, B. A. Gümüş, and H. J. Sloane. 2012. "Modern and Early-Middle Holocene Shells of the Freshwater Mollusc *Unio*, From Çatalhöyük in the Konya Basin, Turkey: Preliminary Palaeoclimatic Implications From Molluscan Isotope Data." *Journal of Archaeological Science* 39: 76–83. https://doi.org/10.1016/j.jas.2011.09.003.

Bates, D., M. Machler, B. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67: 1–48.

Błażejowski, B., G. Racki, P. Gieszcz, K. Małkowski, A. Kin, and K. Krzywiecka. 2013. "Comparative Oxygen and Carbon Isotopic Records of Miocene and Recent Lacustrine Unionid Bivalves From Poland." *Geological Quarterly* 57: 113–122. https://doi.org/10.7306/gq.1072.

BRIG. 2007. "Report on the Species and Habitat Review." JNCC.

Buskirk, B. L., J. Bourgeois, H. W. Meyer, and E. A. Nesbitt. 2016. "Freshwater Molluscan Fauna From the Florissant Formation, Colorado: Paleohydrologic Reconstruction of a Latest Eocene Lake." *Canadian Journal of Earth Sciences* 53: 630–643. https://doi.org/10.1139/cjes-2015-0168.

Butitta, V. L., A. L. Rypel, and E. H. Stanley. 2021. "Environmental Controls on Long-Term Growth of Freshwater Mussels in an Oligotrophic Lake." *Freshwater Science* 40: 316–327. https://doi.org/10.1086/714596.

Çakirlar, C., and R. Şeşen. 2013. "Reading Between the Lines: $\delta^{18}O$ and $\delta^{13}C$ Isotopes of *Unio elongatulus* Shell Increments as Proxies for Local Palaeoenvironments in Mid-Holocene Northern Syria." *Archaeological and Anthropological Sciences* 5: 85–94. https://doi.org/10.1007/s12520-013-0125-8.

Cameron, N. 2024. "Diatoms." In *Must Farm Pile-Dwelling Settlement: Volume 2. Specialist Reports*, edited by R. Ballantyne, A. Cooper, D. Gibson, M. Knight, and I. Robinson Zeki, 57–64. Cambridge: McDonald Institute for Archaeological Research. https://doi.org/10.17863/CAM.106698.

Checa, A. 2000. "A New Model for Periostracum and Shell Formation in Unionidae (Bivalvia, Mollusca)." *Tissue and Cell* 32: 405–416. https://doi.org/10.1054/tice.2000.0129.

Ćmiel, A. M., K. Zając, A. M. Lipińska, and T. Zając. 2019. "Is *Pseudanodonta complanata* the Most Vulnerable of Widespread European Species of Unionids? An Intense Stress Test Leading to a Massive Die-Off." *Aquatic Conservation: Marine and Freshwater Ecosystems* 29: 2185–2192. https://doi.org/10.1002/aqc.3216.

Collins, J., C. F. T. Andrus, R. J. Scott, A. Moe-Hoffman, and E. Peacock. 2020. "Refit and Oxygen Isotope Analysis of Freshwater Mussel Shells From the Tillar Farms Site (3DR30), Southeast Arkansas." *Midcontinental Journal of Archaeology* 45: 39–63. https://doi.org/10.2307/26904359.

Cuttelod, A., M. Seddon, and E. Neubert. 2011. *European Red List of Non-Marine Molluscs*. Luxembourg: Publications Office of the European Union.

Czerniejewski, P., J. Dąbrowski, W. Wawrzyniak, A. Brysiewicz, and O. Surma. 2021. "Shell Morphology, Growth and Longevity of *Unio tumidus* (Bivalvia: Unionidae) From an Archaeological Site and Contemporary Population Inhabiting the Oder Estuary." *Hydrobiologia* 848: 3555–3569. https://doi.org/10.1007/s10750-021-04610-0.

Dettman, D. L., A. K. Reische, and K. C. Lohmann. 1999. "Controls on the Stable Isotope Composition of Seasonal Growth Bands in Aragonitic Fresh-Water Bivalves (Unionidae)." *Geochimica et Cosmochimica Acta* 63: 1049–1057. https://doi.org/10.1016/S0016-7037(99)00020-4.

DuBose, T. P., M. A. Patten, A. S. Holt, and C. C. Vaughn. 2022. "Latitudinal Variation in Freshwater Mussel Potential Maximum Length in Eastern North America." *Freshwater Biology* 67: 1020–1034. https://doi.org/10.1111/fwb.13898.

Dunca, E., B. R. Schöne, and H. Mutvei. 2005. "Freshwater Bivalves Tell of Past Climates: But How Clearly Do Shells From Polluted Rivers Speak?" *Palaeogeography, Palaeoclimatology, Palaeoecology* 228: 43–57. https://doi.org/10.1016/j.palaeo.2005.03.050.

Dzierżyńska-Białończyk, A., Ł. Jermacz, T. Maćkiewicz, J. Gajewska, and J. Kobak. 2018. "Mechanisms and Impact of Differential Fouling of the Zebra Mussel *Dreissena polymorpha* on Different Unionid Bivalves." *Freshwater Biology* 63: 687–699. https://doi.org/10.1111/fwb.13107.

Environment Agency. 2019a. "Water Quality Archive: Kings Dyke Stanground Sluice." https://environment.data.gov.uk/water-quality/view/samplingpoint/AN-53M01.

Environment Agency. 2019b. "Water Quality Archive: R.Nene Peterborough Town Bridge." https://environment.data.gov.uk/water-quality/view/sampling-point/AN-NENE610P.

Evans, C. 2015. "Wearing Environment and Making Islands: Britain's Bronze Age Inland North Sea." *Antiquity* 89: 1110–1124. https://doi.org/10.15184/aqy.2015.99.

French, C. 2024. "Micromorphology." In *Must Farm Pile-Dwelling Settlement: Volume 2. Specialist Reports*, edited by R. Ballantyne, A. Cooper, D. Gibson, M. Knight, and I. Robinson Zeki, 101–108. Cambridge: McDonald Institute for Archaeological Research. https://doi.org/10.17863/CAM.106698.

Fritts, A. K., M. W. Fritts, W. R. Haag, J. A. DeBoer, and A. F. Casper. 2017. "Freshwater Mussel Shells (Unionidae) Chronicle Changes in a North American River Over the Past 1000 Years." *Science of the Total*

*Environment* 575: 199–206. https://doi.org/10.1016/j.scitotenv.2016.09.225.

Grace, M., H. R. Akçakaya, E. Bennett, et al. 2019. "Using Historical and Palaeoecological Data to Inform Ambitious Species Recovery Targets." *Philosophical Transactions of the Royal Society B: Biological Sciences Royal Society* 374: 20190297. https://doi.org/10.1098/rstb.2019.0297.

Haag, W. R., and A. L. Rypel. 2011. "Growth and Longevity in Freshwater Mussels: Evolutionary and Conservation Implications." *Biological Reviews* 86: 225–247. https://doi.org/10.1111/j.1469-185X.2010.00146.x.

Hastie, L. C., M. R. Young, and P. J. Boon. 2000. "Growth Characteristics of Freshwater Pearl Mussels, *Margaritifera margaritifera* (L.)." *Freshwater Biology* 43: 243–256. https://doi.org/10.1046/j.1365-2427.2000.00544.x.

Hornbach, D. J., D. C. Allen, M. C. Hove, and K. R. MacGregor. 2018. "Long-Term Decline of Native Freshwater Mussel Assemblages in a Federally Protected River." *Freshwater Biology* 63: 243–263. https://doi.org/10.1111/fwb.13055.

IPCC. 2014. "Climate Change 2014: Synthesis Report." Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva.

Kaandorp, R. J. G., F. P. Wesselingh, and H. B. Vonhof. 2006. "Ecological Implications From Geochemical Records of Miocene Western Amazonian Bivalves." *Journal of South American Earth Sciences* 21: 54–74. https://doi.org/10.1016/j.jsames.2005.07.012.

Keeling, C. D. 1979. "The Suess Effect: $^{13}$Carbon–$^{14}$Carbon Interrelations." *Environment International* 2: 229–300. https://doi.org/10.1016/0160-4120(79)90005-9.

Kelemen, Z., D. P. Gillikin, L. E. Graniero, et al. 2017. "Calibration of Hydroclimate Proxies in Freshwater Bivalve Shells From Central and West Africa." *Geochimica et Cosmochimica Acta* 208: 41–62. https://doi.org/10.1016/j.gca.2017.03.025.

Kendall, N. W., H. B. Rich, L. R. Jensen, and T. P. Quinn. 2010. "Climate Effects on Inter-Annual Variation in Growth of the Freshwater Mussel (*Anodonta beringiana*) in an Alaskan Lake." *Freshwater Biology* 55: 2339–2346. https://doi.org/10.1111/j.1365-2427.2010.02447.x.

Kimura, D. K. 1980. "Likelihood Methods for the von Bertalanffy Growth Curve." *Fishery Bulletin—National Oceanic and Atmospheric Administration* 77: 765–776.

Klippel, W. E., G. Celmer, and J. R. Purdue. 1978. "The Holocene Naiad Record at Rodgers Shelter in the Western Ozark Highland of Missouri." *Plains Anthropologist* 23: 257–272. https://doi.org/10.1080/2052546.1978.11908892.

Knight, M., R. Ballantyne, M. Brudenell, A. Cooper, D. Gibson, and I. Robinson Zeki. 2024. *Must Farm Pile-Dwelling Settlement: Volume 1. Landscape, Architecture and Occupation*. Cambridge: McDonald Institute for Archaeological Research. https://doi.org/10.17863/CAM.106697.

Kukolich, S., and D. Dettman. 2021. "Reconstructing Seasonal and Baseline Nitrogen Isotope Ratios in Riverine Particulate Matter Using Freshwater Mussel Shells." *Geochemistry, Geophysics, Geosystems* 22: e2020GC009239. https://doi.org/10.1029/2020GC009239.

Langdon, C. 2024. "Chironomids." In *Must Farm Pile-Dwelling Settlement: Volume 2. Specialist Reports*, edited by R. Ballantyne, A. Cooper, D. Gibson, M. Knight, and I. Robinson Zeki, 101–108. Cambridge: McDonald Institute for Archaeological Research. https://doi.org/10.17863/CAM.106698.

Lenth, R. V. 2024. "emmeans: Estimated Marginal Means, Aka Least-Squares Means." https://CRAN.R-project.org/package=emmeans.

Lewandowski, K., and A. Kolodziejczyk. 2014. "Long-Term Changes in the Occurrence of Unionid Bivalves in a Eutrophic Lake." *Folia Malacologica* 22: 301–309. https://doi.org/10.12657/folmal.022.028.

Lewis, J. P., M. J. Leng, J. R. Dean, A. Marciniak, D. E. Bar-Yosef Mayer, and X. Wu. 2017. "Early Holocene Palaeoseasonality Inferred From the Stable Isotope Composition of Unio Shells From Çatalhöyük, Turkey." *Environmental Archaeology Routledge* 22: 79–95. https://doi.org/10.1080/14614103.2015.1116216.

Loftus, E., K. Rogers, and J. Lee-Thorp. 2015. "A Simple Method to Establish Calcite:Aragonite Ratios in Archaeological Mollusc Shells." *Journal of Quaternary Science* 30: 731–735. https://doi.org/10.1002/jqs.2819.

Lopes-Lima, M., L. E. Burlakova, A. Y. Karatayev, K. Mehler, M. Seddon, and R. Sousa. 2018. "Conservation of Freshwater Bivalves at the Global Scale: Diversity, Threats and Research Needs." *Hydrobiologia* 810: 1–14. https://doi.org/10.1007/s10750-017-3486-7.

Lopes-Lima, M., J. Reis, M. G. Alvarez, et al. 2023. "The Silent Extinction of Freshwater Mussels in Portugal." *Biological Conservation* 285: 110244. https://doi.org/10.1016/j.biocon.2023.110244.

Lopes-Lima, M., R. Sousa, J. Geist, et al. 2017. "Conservation Status of Freshwater Mussels in Europe: State of the Art and Future Challenges." *Biological Reviews* 92: 572–607. https://doi.org/10.1111/brv.12244.

Lundquist, S. P., T. A. Worthington, and D. C. Aldridge. 2019. "Freshwater Mussels as a Tool for Reconstructing Climate History." *Ecological Indicators* 101: 11–21. https://doi.org/10.1016/j.ecolind.2018.12.048.

McConnaughey, T. A., and D. P. Gillikin. 2008. "Carbon Isotopes in Mollusk Shell Carbonates." *Geo-Marine Letters* 28: 287–299. https://doi.org/10.1007/s00367-008-0116-4.

Meira, A., J. E. Byers, and R. Sousa. 2024. "A Global Synthesis of Predation on Bivalves." *Biological Reviews* 99: 1015–1057. https://doi.org/10.1111/brv.13057.

Miller, A. C., and B. S. Payne. 1988. "The Need for Quantitative Sampling to Characterize Size Demography and Density of Freshwater Mussel Communities." *American Malacological Bulletin* 6: 49–54.

Mitchell, J. 2018. "Prehistoric Molluscan Faunas of the Yazoo River, Mississippi, USA: Archaeological Perspectives for Modern Conservation." *Environmental Archaeology Routledge* 23: 177–186. https://doi.org/10.1080/14614103.2017.1288886.

Mitchell, J., and M. R. Childress. 2021. "Applying Zooarchaeology: Environmental and Ecological Perspectives From a Prehistoric Freshwater Mussel Assemblage on the Yazoo River, Western Mississippi, USA." *Archaeological and Anthropological Sciences* 13: 88. https://doi.org/10.1007/s12520-021-01334-z.

Mitchell, J., M. R. Childress, and T. W. French. 2023. "Mollusc Remains From an Archaeological Rock Shelter Site on the Buffalo National River, Arkansas, Southeastern USA." *Environmental Archaeology Routledge*: 1–15. https://doi.org/10.1080/14614103.2023.2182978.

Mitchell, J., and E. Peacock. 2014. "A Prehistoric Freshwater Mussel Assemblage From the Big Sunflower River, Sunflower County, Mississippi." *Southeastern Naturalist* 13: 626–638. https://doi.org/10.1656/058.013.0321.

Modesto, V., M. Ilarri, A. T. Souza, et al. 2018. "Fish and Mussels: Importance of Fish for Freshwater Mussel Conservation." *Fish and Fisheries* 19: 244–259. https://doi.org/10.1111/faf.12252.

Müller, T., A. M. Labecka, K. Zając, and M. Czarnoleski. 2021. "Growth Patterns of the Pan-European Freshwater Mussel, *Anodonta anatina* (Linnaeus, 1758) (Bivalvia: Unionidae), Vary With Sex and Mortality in Populations." *Ecology and Evolution* 11: 2907–2918. https://doi.org/10.1002/ece3.7250.

Nakamura, K., R. Sousa, and F. Mesquita-Joanes. 2023. "Collapse of Native Freshwater Mussel Populations: Prospects of a Long-Term Study." *Biological Conservation* 279: 109931. https://doi.org/10.1016/j.biocon.2023.109931.

Nelson, G. A. 2019. "Fishmethods: Fishary Science Methods and Models." https://cran.r-project.org/package=fishmethods.

Nielsen, J. K., and S. Helama. 2021. "Death Assemblages of the Freshwater Mussels *Unio crassus* and *U. tumidus* (Bivalvia, Unionidae) From Southern Finland: Comparing Taphonomical Data With $^{14}C$ Dates." *Bulletin of Geosciences* 96: 459–480. https://doi.org/10.3140/bull.geosci.1798.

Ollard, I., and D. C. Aldridge. 2023. "Declines in Freshwater Mussel Density, Size and Productivity in the River Thames Over the Past Half Century." *Journal of Animal Ecology* 92: 112–123. https://doi.org/10.1111/1365-2656.13835.

Ollard, I., R. Ballantyne, and D. C. Aldridge. 2024. "Research Data Supporting Freshwater Mussel (*Unio pictorum*) Shells Reveal Hydrological and Environmental Change From 1300 BC to the Present Day." Apollo Digital Repository. https://doi.org/10.17863/CAM.108461.

Ożgo, M., M. Urbańska, P. Hoos, et al. 2020. "Invasive Zebra Mussel (*Dreissena polymorpha*) Threatens an Exceptionally Large Population of the Depressed River Mussel (*Pseudanodonta complanata*) in a Postglacial Lake." *Ecology and Evolution* 10: 4918–4927. https://doi.org/10.1002/ece3.6243.

Pauly, D. 1995. "Anecdotes and the Shifting Baseline Syndrome of Fisheries." *Trends in Ecology & Evolution* 10: 430. https://doi.org/10.1016/S0169-5347(00)89171-5.

Peacock, E. 2000. "Assessing Bias in Archaeological Shell Assemblages." *Journal of Field Archaeology* 27: 183–196. https://doi.org/10.1179/jfa.2000.27.2.183.

Peacock, E., J. Mitchell, and C. Jenkins. 2016. "Pre-Columbian Freshwater Mussel Assemblages From the Tallahatchie River in the Lower Mississippi River Alluvial Basin, U.S.A." *American Malacological Bulletin American Malacological Society* 34: 121–132. https://doi.org/10.4003/006.034.0208.

Peacock, E., and J. L. Seltzer. 2008. "A Comparison of Multiple Proxy Data Sets for Paleoenvironmental Conditions as Derived From Freshwater Bivalve (Unionid) Shell." *Journal of Archaeological Science* 35: 2557–2565. https://doi.org/10.1016/j.jas.2008.04.006.

Pfister, L., F. Thielen, E. Deloule, et al. 2018. "Freshwater Pearl Mussels as a Stream Water Stable Isotope Recorder." *Ecohydrology* 11: e2007. https://doi.org/10.1002/eco.2007.

Pickard, C., A. Boroneant, and C. Bonsall. 2017. "Molluscan Remains From Early to Middle Holocene Sites in the Iron Gates Reach of the Danube, Southeast Europe." In *Molluscs in Archaeology: Methods, Approaches and Applications*, edited by M. J. Allen, 179–194. Oxford: Oxbow Books.

Popejoy, T., C. R. Randklev, S. Wolverton, and L. Nagaoka. 2018. "Conservation Implications of Late Holocene Freshwater Mussel Remains of the Leon River in Central Texas." *Hydrobiologia* 810: 477–487. https://doi.org/10.1007/s10750-016-3041-y.

R Core Team. 2022. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Randklev, C. R., S. Wolverton, B. Lundeen, and J. H. Kennedy. 2010. "A Paleozoological Perspective on Unionid (Mollusca: Unionidae) Zoogeography in the Upper Trinity River Basin, Texas." *Ecological Applications* 20: 2359–2368. https://doi.org/10.1890/09-1425.1.

Rose, M., and D. Balbi. 1997. *Rivers Nene and Great Ouse Eutrophication Studies: Final Report*. Peterborough: Environment Agency.

Rubino, M., D. M. Etheridge, C. M. Trudinger, et al. 2013. "A Revised 1000 Year Atmospheric $\delta^{13}C$-$CO_2$ Record From Law Dome and South Pole, Antarctica." *Journal of Geophysical Research: Atmospheres* 118: 8482–8499. https://doi.org/10.1002/jgrd.50668.

Rypel, A. L., W. R. Haag, and R. H. Findlay. 2008. "Validation of Annual Growth Rings in Freshwater Mussel Shells Using Cross Dating." *Canadian Journal of Fisheries and Aquatic Sciences* 65: 2224–2232. https://doi.org/10.1139/F08-129.

Schöll-Barna, G., A. Demény, G. Serlegi, et al. 2012. "Climatic Variability in the Late Copper Age: Stable Isotope Fluctuation of Prehistoric *Unio pictorum* (Unionidae) Shells From Lake Balaton (Hungary)." *Journal of Paleolimnology* 47: 87–100. https://doi.org/10.1007/s10933-011-9561-6.

Schöne, B. R., E. Dunca, H. Mutvei, and U. Norlund. 2004. "A 217-Year Record of Summer Air Temperature Reconstructed From Freshwater Pearl Mussels (*M. margaritifera*, Sweden)." *Quaternary Science Reviews* 23: 1803–1816. https://doi.org/10.1016/j.quascirev.2004.02.017.

Schöne, B. R., N. A. Page, D. L. Rodland, et al. 2007. "ENSO-Coupled Precipitation Records (1959–2004) Based on Shells of Freshwater Bivalve Mollusks (*Margaritifera falcata*) From British Columbia." *International Journal of Earth Sciences* 96: 525–540. https://doi.org/10.1007/s00531-006-0109-3.

Seddon, M. B., I. J. Killeen, and A. P. Fowles. 2014. "A Review of the Non-Marine Mollusca of Great Britain: Species Status No. 17." Countryside Council for Wales.

Sousa, R., F. Pilotto, and D. C. Aldridge. 2011. "Fouling of European Freshwater Bivalves (Unionidae) by the Invasive Zebra Mussel (*Dreissena polymorpha*)." *Freshwater Biology* 56: 867–876. https://doi.org/10.1111/j.1365-2427.2010.02532.x.

Strayer, D. L. 2008. *Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance*. Berkeley: University of California Press.

Strayer, D. L., S. K. Hamilton, and H. M. Malcom. 2021. "Long-Term Increases in Shell Thickness in *Elliptio complanata* (Bivalvia: Unionidae) in the Freshwater Tidal Hudson River." *Freshwater Biology* 66: 1375–1381. https://doi.org/10.1111/fwb.13723.

Timberlake, C. 2024. "Ostracods." In *Must Farm Pile-Dwelling Settlement: Volume 2. Specialist reports*, edited by R. Ballantyne, A. Cooper, D. Gibson, M. Knight, and I. Robinson Zeki, 65–78. Cambridge: McDonald Institute for Archaeological Research. https://doi.org/10.17863/CAM.106698.

Tyers, I., P. Marshall, C. Bronk Ramsey, et al. 2024. "Chronology." In *Must Farm Pile-Dwelling Settlement: Volume 2. Specialist Reports*, edited by R. Ballantyne, A. Cooper, D. Gibson, M. Knight, and I. Robinson Zeki, 1265–1281. Cambridge: McDonald Institute for Archaeological Research. https://doi.org/10.17863/CAM.106698.

van Plantinga, A. A., and E. L. Grossman. 2018. "Stable and Clumped Isotope Sclerochronologies of Mussels From the Brazos River, Texas (USA): Environmental and Ecologic Proxy." *Chemical Geology* 502: 55–65. https://doi.org/10.1016/j.chemgeo.2018.10.012.

Vaughn, C. C., C. M. Taylor, and K. J. Eberhard. 1997. "A Comparison of the Effectiveness of Timed Searches vs. Quadrat Sampling in Mussel Surveys." In *Conservation and Management of Freshwater Mussels II: Proceedings of a UMRCC Symposium*, 157–162.

Versteegh, E. A. A., S. R. Troelstra, H. B. Vonhof, and D. Kroon. 2009. "Oxygen Isotope Composition of Bivalve Seasonal Growth Increments and Ambient Water in the Rivers Rhine and Meuse." *PALAIOS* 24: 497–504. https://doi.org/10.2110/palo.2008.p08-071r.

Versteegh, E. A. A., H. B. Vonhof, S. R. Troelstra, R. J. G. Kaandorp, and D. Kroon. 2010. "Seasonally Resolved Growth of Freshwater Bivalves Determined by Oxygen and Carbon Isotope Shell Chemistry." *Geochemistry, Geophysics, Geosystems* 11. https://doi.org/10.1029/2009GC002961.

Vickers, K., H. Kenward, and L. Girvan. 2024. "Coleoptera." In *Must Farm pile-dwelling settlement: Volume 2. Specialist Reports*, edited by R. Ballantyne, A. Cooper, D. Gibson, M. Knight, and I. Robinson Zeki, 79–99. Cambridge: McDonald Institute for Archaeological Research. https://doi.org/10.17863/CAM.106698.

von Bertalanffy, L. 1938. "A Quantitative Theory of Organic Growth (Inquires on Growth Laws. II)." *Human Biology* 10: 181–213.

Vonhof, H. B., J. C. A. Joordens, M. L. Noback, J. H. J. L. van der Lubbe, C. S. Feibel, and D. Kroon. 2013. "Environmental and Climatic Control on Seasonal Stable Isotope Variation of Freshwater Molluscan Bivalves in the Turkana Basin (Kenya)." *Palaeogeography, Palaeoclimatology, Palaeoecology* 383–384: 16–26. https://doi.org/10.1016/j.palaeo.2013.04.022.

Wolverton, S., C. R. Randklev, and J. H. Kennedy. 2010. "A Conceptual Model for Freshwater Mussel (Family: Unionidae) Remain Preservation in Zooarchaeological Assemblages." *Journal of Archaeological Science* 37: 164–173. https://doi.org/10.1016/j.jas.2009.09.028.

Yoshimura, T., H. Izumida, R. Nakashima, et al. 2015. "Stable Carbon Isotope Values in Dissolved Inorganic Carbon of Ambient Waters and Shell Carbonate of the Freshwater Pearl Mussel (*Hyriopsis* sp.)." *Journal of Paleolimnology* 54: 37–51. https://doi.org/10.1007/s10933-015-9834-6.

Zeileis, A., and T. Hothorn. 2002. "Diagnostic Checking in Regression Relationships." *R News* 2: 7–10.

Zieritz, A., and D. C. Aldridge. 2009. "Identification of Ecophenotypic Trends Within Three European Freshwater Mussel Species (Bivalvia: Unionoida) Using Traditional and Modern Morphometric Techniques." *Biological Journal of the Linnean Society* 98: 814–825. https://doi.org/10.1111/j.1095-8312.2009.01329.x.

**Supporting Information**

Additional supporting information can be found online in the Supporting Information section.