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7 **Freshwater mussels as a tool for reconstructing climate history**

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Abstract

Sclerochronology provides an important and widely used tool to examine annual environmental patterns in marine systems, but few similar tools have been developed to establish ecological indicators in freshwaters on a seasonal scale. Previous work using marine mussels as ecological indicators have practiced shell ashing, acetate peels, and thin sectioning, all of which destroy the specimens. We studied the external annual rings of three freshwater mussel species with clear annual bands from the River Medway, UK, in order to non-invasively investigate the relationship between banding patterns, growth parameters, location, and seasonal water temperatures. We tested the accuracy and precision of this method through repetition of measurements and the reproduction of results through separate agers. Overall, mussels living downstream had higher length-at-age curves, asymptotic lengths (L_{∞}), and growth constants (k) than those living upstream. In *Unio pictorum* and *Unio tumidus*, there was a negative relationship between asymptotic lengths and growth constants, indicating that larger asymptotic lengths took more time to reach. *Unio tumidus* had the highest asymptotic lengths and the lowest growth constants across sites while *Unio pictorum* had the lowest asymptotic lengths and the highest growth constants across sites. *Anodonta anatina* showed simultaneously increasing asymptotic lengths with increasing growth constants and had the highest overall lengths of all three species. Summer water temperatures had the largest positive effect on annual growth, followed by spring and winter water temperatures, while autumnal temperatures had a negative impact on growth. The findings of this study suggest that through their annual banding, freshwater mussels can be used as tools to document historical water temperatures, especially in the warmer months of spring and summer, and can therefore serve as powerful indicators of spatial and temporal patterns in water temperature. Such tools can help infer palaeoclimatic conditions from fossil and subfossil

shells, establish baselines for understanding future climatic change, and support conservation efforts aimed at protecting temperature-sensitive taxa.

Keywords

Ecological indicator; Sclerochronology; Unionidae; Climate change; Freshwater ecosystems; Seasonality

1. Introduction

Freshwater ecosystems are facing a ‘global crisis’ (Strayer and Dudgeon, 2010) as a result of increasing human demands (i.e. irrigation, potable supplies, aquaculture, industrial uses, and sanitation) combined with greater fragmentation, impoundment, pollution, introduction of non-native species and effects of climate change (Johnson and Evans, 1990; Vörösmarty et al., 2000; Strayer and Dudgeon, 2010). Climate change, in particular, affects freshwaters species more than marine or terrestrial species since freshwater temperatures are highly dependent on air temperatures, and habitats are often fragmented, limiting the ability of species to move as the environment changes (Jenkins, 2003; Woodward et al., 2010). It is therefore essential that we develop effective tools for monitoring freshwater ecosystems, identifying habitat degradation and species decline, and quantifying the effects of rehabilitation programmes. Currently, we are lacking sufficient freshwater temperature records, and there is a need to establish proxies to reconstruct past temperature fluctuations and to set baselines against which future change can be compared. These proxies can be used to assess freshwater quality and then potentially create predictive models (Alizadeh, et al., 2017; Wang, et al., 2014). This will allow us to understand how freshwater communities have responded to climate change in the past and how they might be affected in the future.

Common aquatic animals used as environmental proxies include corals, brachiopods, fish, and bivalves (Hudson et al., 1976; Helama et al., 2006; Scourse et al., 2006). Freshwater mussels (Bivalvia: Unionida) have been identified as effective indicator taxa whose abundance is often positively correlated with associated biodiversity (Aldridge et al., 2007; Chowdhury et al., 2016). Their capacity to filter large volumes of water (up to 40 litres per individual per day (Tankersley

and Dimock Jr., 1993)) means that their growth patterns are likely to capture information about their surrounding environment.

Annual growth bands of living organisms have been used as ecological indicators in taxa as diverse as trees, fishes and corals. Studies have encompassed terrestrial, marine and freshwater ecosystems and covered geographies including polar, temperate and tropical regions. The greatest attention has focused on tree rings as climatic proxies. By relating spatial variation in annual tree rings to anomalies in annual climate records, dendrochronology can provide a clear picture of recent (Douglass, 1941) and palaeoclimatic (LaMarche, 1974) patterns over long periods of time (Cook et al., 1995). Proxies have also been created for environmental and climatic records of aquatic systems using a similar technique called sclerochronology. The annual increments in fish otoliths and scales, coral skeletons, and bivalve shells can be measured and cross-dated to provide information on factors such as climate, hydrology, pollution, reproduction and migration patterns (Helama et al., 2006; Rypel et al., 2008; Helmle and Dodge, 2011).

Annual bands on bivalve mollusc shells have become widely used as a tool for ageing and reconstructing population structure. Rings are counted from the shell margin towards the umbo, linking them with growth years (Lefevre and Curtis, 1912; Jones, 1983). Though genetic factors can affect early growth increments of molluscs, external factors mainly drive the visible, annual growth banding on mussel shells (Coker et al., 1921; Marchitto, Jr. et al., 2000; Gooding et al., 2009). Thus, growth bands are good indicators of environmental parameters including temperature (Davenport, 1938; Kennish and Olsson, 1975; Helama et al., 2006), salinity (Navarro, 1988; Marsden and Pilkington, 1996), nutrients (Ansell, 1968; Schöne et al., 2003) and dissolved oxygen (Goodwin et al., 2001). Molluscs develop these dark bands due to a discontinuity in growth and a withdrawing of the mantle during stress or large disturbances (such

as colder temperatures each winter). When molluscs start to grow again, they do not start exactly where they left off, but a little distance back, resulting in a slight overlap in the outer layers of the shell which forms a dark band.

Annual banding of marine bivalves, specifically *Arctica islandica* and *Spisula solidissima*, has widely been studied for the purpose of sclerochronology and cross-matching (Jones, 1983; Marchitto, Jr. et al., 2000; Schöne et al., 2002; Scourse et al., 2006), whereas very few studies have looked at the potential of freshwater bivalve banding and growth patterns as environmental indicators. Freshwater mussels represent an attractive tool for environmental monitoring due to their clear annual rings (Neves and Moyer, 1988; Haag and Commens-Carson, 2008; Rypel et al., 2008), longevity (Aldridge, 1999), wide geographical distribution (Lopes-Lima et al., 2016), high abundance and accessibility for sampling (Strayer and Smith, 2003), and opportunity for non-destructive ageing of live specimens (Aldridge, 1999; Helama and Nielsen, 2008; Rypel et al., 2008).

Freshwater mussels have been effectively used to interpret a variety ecological and hydrological patterns, but to date have not been used to investigate patterns in seasonal climate history. For example, European unionid mussels have been used to study the effects of habitat, hydrology, and sediment composition on shell morphology (Zieritz and Aldridge, 2009), to determine the effect of genetic differentiation on ecophenotypic differences (Zieritz et al., 2010), to compare shell morphology and population dynamics in a sympatric population (Aldridge, 1999), and to compare the density of freshwater mussels to overall taxon richness (Aldridge et al., 2007).

Studies of growth rates in freshwater mussels of the Illinois River, USA, demonstrate a general increasing trend over the past 150 years which has been attributed to impoundment, eutrophication, water quality improvements, and warming water temperatures (Fritts et al.,

2017). However, it is likely that local habitat effects and species-specific responses have additional effects on growth patterns. Furthermore, it can be expected that environmental effects will have a differing influence on growth rates depending on the time of the year, which may render seasonal pattern studies more informative than annual studies. For example, a cold winter or hot summer can suppress bivalve growth, but a warmer spring can facilitate growth (Killam and Clapham, 2018).

In this study, we investigated the utility of growth bands as climatic indicators in three widespread European freshwater mussel species, *Unio pictorum* (Linnaeus), *Unio tumidus* (Philipsson) and *Anodonta anatina* (Linnaeus). These mussels were chosen based on their International Union for the Conservation of Nature (IUCN) status of “least concern” (IUCN, 2018), their abundance and widespread distribution in the River Medway, and their clear annual growth rings. Other species native to the UK and present in the River Medway were less suited for our study; *Pseudanodonta complanata* (Rossmässler), is of conservation concern (IUCN, 2018), while *Anodonta cygnea* (Linnaeus) is rare and localised within the River Medway (Aldridge, 2009).

Most previous work on bivalve growth rings have resorted to sectioning shells or sectioning hinge ligaments (Neves and Moyer, 1998; Cerrato, 2000; Goodwin et al., 2001). These approaches are necessary for species that are slow-growing, long-lived or subject to erosion of the external shell. However, the external rings of unionid mussels are typically clear and intact, allowing us to determine age without killing or damaging the specimens. Furthermore, the three species that we used have been successfully aged by their external annuli in previous studies due to their fast growth, short lives, and little umbonal erosion (Aldridge, 1999; Zieritz and Aldridge, 2009).

Shell annulus patterns along the length of a river were investigated to understand interspecific and habitat-induced effects. It was anticipated that growth rates in these filter feeders would increase towards the lower reaches of the river continuum due to increased temperature and greater abundance of particulate organic matter and algae (Vannote et al., 1980). The three objectives of our study were: (1) to compare mussel growth banding between different species and different locations along the river; (2) to assess the relationship between band spacing and annual water temperatures; and (3) to investigate the effects of seasonal climate on mussel growth, and therefore establish the use of mussel bands to document past climate changes.

2. Material and Methods

2.1 Study site

Studies were conducted at three sites within a 27 km navigable reach of the River Medway, Kent, UK (Fig. 1), encompassing the entire range of abundant mussel populations within the river. The most upstream site was located in Haysden (51.19° N, 0.25° E) at the upper navigational limit of the river. The mid site was located 17 km downstream in Watlington (51.25° N, 0.42° E) and the lowest site was located in Ringlestone (51.27° N, 0.69° E), approximately 1 km upstream from the river's tidal sluice. The study reach is a highly regulated lowland river, comprising 10 locks and a benthos of gravels and marginal silt. Maximum width ranged from 15 m to 30 m and depth from <2 m to <4 m. Littoral vegetation was dominated by *Nuphar lutea* L. The study reach is unusual in supporting all five UK unionid mussel species: *P. complanata*, *A. cygnea*, *A. anatina*, *U. pictorum* and *U. tumidus* (Aldridge, 2009).

Fig. 1. Distribution of the three sampling locations on the River Medway, Kent, UK. The four surrounding counties in Kent are listed in bold.

2.2 Mussel collection and measurement of shell growth rings

In October 2017, approximately 30 individuals from each of three species of mussels were collected from each site by hand sampling. Studies focused on the three most numerically abundant and widespread species in the river (Aldridge, 2009): *A. anatina*, *U. pictorum* and *U.*

tumidus. Annual bands, identified as continuous dark bands around the entire shell (Aldridge, 1999), were measured from each mussel using a Vernier caliper.

2.3 Standardisation and data analysis

Individual growth parameters were determined using the Walford plot model (Walford, 1946; Aldridge, 1999), which uses a linear regression common to de-trending individual growth series (Helama and Nielsen, 2008). Compared to other methods of fitting growth curves, such as the logistic or the Gompertz, this model is convenient for large numbers of individuals (Walford, 1946) and assumes von Bertalanffy growth curves from the equation:

$$L_{t+m} = cL_t + d \quad (1)$$

where L_t is length of shell at time t , L_{t+m} is length of shell at time $t + m$; m is the interval at which measurements were taken (one year in this study, $m=1$), c is the slope of the Walford plot line, and d is the y-intercept of the Walford plot line. The asymptotic length (L_∞) and the growth constant (k) were then calculated for individuals of all three species at all three sites using equations 2 and 3. The theoretical maximum growth (L_∞) of a mussel if allowed to grow to its full potential is determined by the equation:

$$L_\infty = d/(1-c) \quad (2)$$

where L_∞ is the asymptotic length, or the y-intercept of the 45° line. The rate at which the asymptotic length is reached is determined by the equation:

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$$222 \quad k = -\ln(c)/m \quad (3)$$

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224 where k is the growth constant.

225 To account for declining growth increments as mussels age and to plot individual mussel

226 growth trends, the natural logarithm of all the raw growth data was taken (Scourse et al., 2006).

227 Without doing so, the growth data for the most recent growth years would have smaller residuals

228 since the majority of our mussels were at an age where growth had already slowed down

229 significantly. This would have displayed a gradual slow of annual mussel growth over time due

230 to ageing, not changing water parameters. Using similar techniques as is used in tree ring width

231 series, each mussel was plotted as the deviation from a standardised index. The ratios of annual

232 actual-to-expected ring width yields a set of dimensionless ring ‘indices’ with a defined mean of

233 1.0 and an approximately homogeneous variance (Fritts, 1976; Cook et al., 1995). Rather than

234 averaging raw growth data, this method allows us to understand good *versus* bad years for

235 growth since it displays each annulus as a positive or negative deviation from the mean index.

236 This method accounts for the fact that mussels, like trees, grow at a faster rate when young. The

237 index of each mussel was calculated as:

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$$239 \quad I_t = R_t/G_t \quad (4)$$

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241 where R_t is the ring width measured, G_t is the expected ring width based on the slope and y-

242 intercept of its Walford plot, and I_t is the resultant growth ring standardised index with a mean of

243 1.0. This analysis equalised the growth variations regardless of age or size. By plotting the

244 indices of each mussel’s growth increments, a unique mean standardised growth index was

created for each species at all three sites. This ensured differences in growth trends between location and species were documented.

2.4 Temperature

Since seasonal water temperatures are rarely recorded over long time scales, little research exists on how seasonal temperature changes affect mussel growth. Using logistic modelling based on seasonal air temperatures recorded from the Environment Agency (EA) and Meteorological Office (Met Office), we filled in missing water temperature data to perform seasonal growth analyses. In order to isolate the effects of temperature from the various other factors affecting growth, we also included sex, location, and species in our analyses. Although there are many other driving factors, literature reviews and previous research led us to conclude that temperature is the main driving factor for most other water parameters and is therefore the factor that would supply the most valuable information (Domisch et al., 2013; Mulholland et al., 1997; Woodward et al., 2010).

Regular long-term data on water temperature within the River Medway was not available (hence, the need to establish proxies), and since we started our study in 2017 and used annuli dating back to 2009, we did not have the ability to record all the necessary water temperatures directly. However, water temperature for shallow rivers such as the Medway have been shown to closely track air temperature, especially when averaged across seasons (Webb and Nobilis, 1999). Daily air temperature records from 2009 to 2016 were acquired from the UK Meteorological Office for the central England district (Met Office, 2018) along with irregularly recorded water temperature data for the same period from the UK Environment Agency

(Environment Agency, 2018) at six monitoring stations identified as being closest to the study sites. Water temperature was related to air temperature using a logistic model of the form:

$$T_w = \frac{\alpha}{1 + e^{\gamma(\beta - T_a)}} \quad (5)$$

where T_w is the water temperature, T_a the air temperature, α the estimate of the highest water temperature, β the air temperature at the inflection point and γ the steepest slope of the logistic function (see Benyahya et al., 2007). The coefficients α , β and γ were unknown and were calculated using numerical optimization. We followed the methods of Webb et al. (2003), whereby the root mean square error (RMSE) between the observed and calculated water temperatures was minimised. RMSE measures the predicted values minus the observed values in a quadratic sense and can be impacted by outliers (Janssen and Heuberger 1995). Numerical optimization was undertaken using the ‘optim’ function in R (R Core Team, 2017) using the default Nelder and Mead (1965) method. For each site, 80% of the water temperature data was used to train the model. The remaining 20% used to examine model fit, the accuracy of the coefficients and transferability, again using RMSE. All logistic models passed the RMSE validation, each with a difference between validation RMSE and training RMSE within ± 0.4 . This allowed daily predicted water temperatures to be calculated in the Medway, which were summarized for each year and for each season: winter (December – February), spring (March – May), summer (June – August) and autumn (September – November). Furthermore, recent literature suggests that the incorporation of both observed and predicted values into the forecasting model improves performance (Alizadeh et al., 2017). Soft-computing techniques using logistic models are becoming more popular in environmental management research (e.g. Olyaie, et al., 2015).

Given that mussels primarily grow between mid-spring and mid-autumn and cease growth during winter (which creates the dark annual band), annual mussel growth measured by the length of each band corresponds to the temperature of the previous year's spring, summer, and autumn and the current year's winter. Therefore, spring, summer, and autumn temperatures were shifted up a year to connect seasonal mean temperatures with their corresponding annual growth indices. For example, a growth ring created in 2012 will demarcate the outer band edge of growth and temperature information for spring 2011, summer 2011, autumn 2011, and winter 2011 (December) going into winter 2012 (January and February).

2.5 Statistical analyses

All statistical analyses were implemented using R programming language (R Core Team, 2017). Analysis of variance (ANOVA) tests were used to determine if length-at-age curves, asymptotic lengths (L_{∞}), or growth constants (k) differed between sites and species. Final models were created after examining Pearson plots of the residuals versus fitted values and Cook's distance. Inspection of the normal Q-Q plots and histograms of the Pearson residuals did not show deviations from homoscedasticity or normality. Where significant differences were identified from the ANOVAs, *post hoc* Tukey's Honestly Significant Difference (HSD) tests were run using the 'multcomp' package (Hothorn et al., 2008) to determine between group differences.

A linear mixed-effects model (LME) was employed to analyse the relationship between standardised mussel growth indices, site, species, and antecedent seasonal temperature. Within the model, seasonal temperature, site, and species (without interaction terms) were entered as fixed effects and individual mussel identity as a random effect. Inspection of residual plots did not reveal deviations from homoscedasticity or normality. The likelihood ratio test was used as a

means to attain p-values for each variable in the linear mixed-effects model. This was conducted by withdrawing each variable individually and running an ANOVA between the null model and each new model excluding one variable. This allowed us to understand the effect of each variable individually. The likelihood ratio test also helped us to determine which variables to include in the final model through Chi-squared distributions and Akaike information criterion (AIC). The LME model was created using the ‘lme4’ package (Bates et al., 2015) and Tukey HSD tests were again run using the ‘multcomp’ package (Hothorn et al., 2008). All significance was assessed at $P < 0.05$.

3. Results

3.1 Length-at-age relationships

Length-at-age curves differed significantly between sites and between species (Table 1a). Overall, mussels showed significantly larger annual lengths at Ringlestone than at Haysden or Wateringbury. Lengths increased moving downstream from Haysden to Wateringbury to Ringlestone. *A. anatina* had significantly higher lengths than either *U. tumidus* or *U. pictorum*, and *U. pictorum* had significantly higher lengths than *U. tumidus* (Fig. 2 and Table 1a).

Overall, asymptotic length (L_{∞}) differed significantly between sites with largest asymptotic lengths downstream at Ringlestone and smallest lengths at Wateringbury, and between species with significantly larger L_{∞} in *U. tumidus* and *A. anatina* compared to *U. pictorum* (Fig. 3a and Table 1b). Growth constant (k) also differed significantly between sites with higher k in Ringlestone and in Wateringbury compared to in Haysden, and between species with higher k in *U. pictorum* and *A. anatina* than in *U. tumidus* (Fig. 3b and Table 1c).

3.2 Standardised growth for different years and seasons

The standardised growth indices showed that growth rates were relatively low across all sites and species in 2012 and 2013, and relatively high in 2015 and 2016 (Fig. 4). There was a strong correlation between annual growth patterns in *A. anatina* and *U. pictorum*, but less similarity between these species and *U. tumidus* (Table 2), suggesting that not all species respond to temperature changes in the same way. At most sites, *A. anatina* and *U. pictorum* grew well in 2011 whereas this was a relatively poor growth year for *U. tumidus*. Conversely, 2014 was a relatively good growth year for *U. tumidus* but a below average year for *A. anatina* and *U. pictorum*.

The linear mixed-effects model (LME) of mussel growth as a function of site (Table 3) showed significantly lower growth at both Ringlestone and Wateringbury compared to at Haysden. Annual growth indices were significantly higher in *U. tumidus* compared to *U. pictorum*. Additional LME models were run separating *U. tumidus* from the other species due to its unique standardised growth trends, but all species showed similar trends in growth as a function of seasonal temperatures and site. Seasonal water temperature was a significant predictor of mussel growth in all species (Table 3 and Fig. 5); warmer water temperatures in the winter, spring, and summer were associated with increased mussel growth, while warmer autumns decreased growth. Summer temperatures had the biggest positive effect on growth, followed by spring, then winter, and finally autumn.

Fig. 2. Mussel length-at-age curves from three sites on the River Medway separated by species; (a) *A. anatina*, (b) *U. pictorum*, (c) *U. tumidus*; triangles represent Haysden, circles represent Watringbury, and squares represent Ringlestone. The points are staggered for clarity. Error bars represent standard error.

(a) Length-at-age ANOVA					
Variable	d.f.	Sum Sq	Mean Sq	F-value	p-value
Site	2	9168	4584	125.10	<0.001*
Species	2	13321	6660	181.70	<0.001*
Year	1	358598	358598	9782.30	<0.001*
Residuals	1723	63162	37		
(b) L_{∞}					
Site	2	10001	5000	3.59	0.030*
Species	2	33710	16855	12.11	<0.001*
Residuals	253	352287	1392		
(c) k					
Site	2	0.135	0.0676	6.16	0.002*
Species	2	0.864	0.4321	39.34	<0.001*
Residuals	253	2.779	0.011		
(a) Length-at-age Tukey HSD					
Variable	Estimate	Std. Error	t-value	p-value	
Ringlestone- Haysden	3.21	0.38	8.46	<0.001*	
Watringbury - Haysden	0.67	0.36	1.87	0.15	
Watringbury- Ringlestone	-2.54	0.35	-7.20	<0.001*	
<i>U. pictorum</i> - <i>A. anatina</i>	-5.27	0.39	-13.47	<0.001*	
<i>U. tumidus</i> - <i>A. anatina</i>	-12.41	0.36	-34.31	<0.001*	
<i>U. tumidus</i> - <i>U. pictorum</i>	-7.15	0.36	-20.09	<0.001*	
(b) L_{∞}					
Ringlestone- Haysden	0.35	6.37	0.06	1	
Watringbury - Haysden	-10.79	5.63	-1.92	0.14	
Watringbury- Ringlestone	-11.14	5.79	-1.93	0.13	
<i>U. pictorum</i> - <i>A. anatina</i>	-16.95	5.90	-2.87	0.01 *	
<i>U. tumidus</i> - <i>A. anatina</i>	10.25	5.92	1.73	0.20	
<i>U. tumidus</i> - <i>U. pictorum</i>	27.20	5.58	4.88	<0.001 *	
(c) k					
Ringlestone- Haysden	0.04	0.02	2.45	0.04*	
Watringbury - Haysden	0.04	0.02	2.42	0.04*	
Watringbury- Ringlestone	-0.01	0.02	-0.34	0.94	
<i>U. pictorum</i> - <i>A. anatina</i>	0.01	0.02	0.78	0.71	
<i>U. tumidus</i> - <i>A. anatina</i>	-0.12	0.02	-6.92	<0.001*	
<i>U. tumidus</i> - <i>U. pictorum</i>	-0.13	0.02	-8.17	<0.001*	

Table 1 Statistical comparisons of mussel growth parameters between sites and species within the River Medway; (a) length-at-age curves, (b) asymptotic length L_{∞} , and (c) growth constant k ; the analysis of variance (ANOVA) tests were run with mean lengths, L_{∞} and k as functions of site and species; corresponding *post hoc* Tukey HSD estimates represent the difference in significant response variables between levels; * indicates $p < 0.05$.

379 **Fig. 3.** Boxplots of the growth parameters for three *Unionidae* species found in three sites along
380 the River Medway; (a) plot of asymptotic lengths L_{∞} and (b) plot of growth constants k ; boxplots
381 annotated with different letters indicate groups that are statistically different ($P < 0.05$); boxes are
382 based on mean, 1st and 3rd quartiles, and error bars represent 95% confidence intervals; dots
383 mark outliers.

Fig. 4. Mean annual standardised growth indices for all records of each species at all three sites; (a) *A. anatina* at Haysden, (b) *U. pictorum* at Haysden, (c) *U. tumidus* at Haysden, (d) *A. anatina* at Watlington, (e) *U. pictorum* at Watlington, (f) *U. tumidus* at Watlington, (g) *A. anatina*

388 at Ringlestone, (h) *U. pictorum* at Ringlestone, (i) *U. tumidus* at Ringlestone; error bars represent
 389 standard error.

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	UpHays	UpWater	UpRingle	AaHays	AaWater	AaRingle	UtHays	UtWater	UtRingle
UpHays	1.00								
UpWater	0.90	1.00							
UpRingle	0.99*	0.90	1.00						
AaHays	0.97*	0.92	0.97*	1.00					
AaWater	0.96*	0.94	0.93	0.94	1.00				
AaRingle	0.86	0.98*	0.84	0.88	0.95*	1.00			
UtHays	0.65	0.90	0.66	0.70	0.73	0.89	1.00		
UtWater	0.47	0.79	0.46	0.51	0.63	0.83	0.94*	1.00	
UtRingle	0.28	0.65	0.27	0.33	0.48	0.71	0.86	0.98*	1.00

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394 **Table 2** Correlation matrix of all mean standardised growth indices of mussels from the River
 395 Medway; Up represents species *U. pictorum*, Aa represents *A. anatina*, Ut represents *U. tumidus*,
 396 Hays represents site Haysden, Water represents Watringbury, and Ringle represents
 397 Ringlestone; * indicates a significant correlation after Bonferroni correction ($P \leq 0.006$).

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Variable	Linear mixed-effects model				Likelihood ratio test			
	Estimate	Std. Error	t-value	p-value	Variable	χ^2	d.f.	p-value
(Intercept)	-0.79	0.09	-8.43	<0.001*				
Summer	0.08	0.005	17.40	<0.001*	Summer	278.76	1	<0.001*
Spring	0.05	0.003	16.02	<0.001*	Spring	239.08	1	<0.001*
Winter	0.05	0.002	20.30	<0.001*	Winter	369.05	1	<0.001*
Autumn	-0.03	0.003	-11.65	<0.001*	Autumn	130.50	1	<0.001*
Ringlestone-Haysden	-0.06	0.004	-13.65	<0.001*	Site	198.05	2	<0.001*
Wateringbury-Haysden	-0.06	0.004	-13.72	<0.001*				
Wateringbury-Ringlestone	-0.0001	0.003	-0.04	1.00				
<i>U. pictorum</i> - <i>A. anatina</i>	-0.0007	0.003	-0.21	0.98	Species	8.51	2	0.01*
<i>U. tumidus</i> - <i>A. anatina</i>	0.007	0.003	2.29	0.06				
<i>U. tumidus</i> - <i>U. pictorum</i>	0.007	0.003	2.57	0.03*				

Table 3 Results from linear mixed-effects model (LME) and likelihood ratio test for the effects of seasonal temperature, site, and species on annual mussel growth in the River Medway; the LME was run with standardized annual mussel growth as the response variable versus all analysed variables as fixed effects: seasonal temperatures, site, and species; estimates for continuous variables (i.e. seasonal temperatures) represent the difference in standardised growth indices as temperatures rise; estimates for discrete variables (i.e. site and species) represent the difference in standardised growth indices between two levels; the likelihood ratio test was run with standardised annual mussel growth as a function of seasonal temperatures, site, and species; * indicates $P < 0.05$.

Fig. 5. Mean annual standardised mussel growth indices (SIs) compared to the corresponding mean seasonal water temperatures in the River Medway; (a) standardised growth indices for all three *Unionidae* species at all three sites over a period of eight years; (b) seasonal water temperatures for each site across the same time period; error bars represent standard error.

4. Discussion

Our data demonstrate that growth banding of freshwater mussels can serve as a powerful indicator of historic water temperatures. In the River Medway, downstream populations were typically larger age-to-age. *U. pictorum* and *A. anatina* shared similar years for above average and below average growth, while *U. tumidus* responded differently. Warmer winters, springs and summers were correlated with wider growth bands and warmer autumns were correlated with narrower growth bands.

4.1 Length-at-age patterns

All three species of mussel had lowest length-at-age growth trends at the upstream river site, Haysden, and higher trends downstream. These patterns were further supported by our finding that downstream mussels had higher asymptotic lengths, indicating that these individuals have the potential to reach larger sizes. Mussels also showed larger growth constants downstream, but these statistics were mainly driven by *A. anatina* from Ringlestone. The relationship between growth constant and the asymptotic length is usually negative so that larger growth constants often correlate with lower asymptotic lengths and indicate that the asymptotic length is reached at a faster rate (Bauer, 1992; Aldridge, 1999). Both *U. pictorum* and *U. tumidus* had lowest growth constants in Ringlestone, meaning they would reach their asymptotic lengths at a slower rate. This could be because all mussels in Ringlestone had greater asymptotic lengths and would therefore need more time to reach their maxima. *U. tumidus* had the highest asymptotic length and the lowest growth constant overall, whereas *U. pictorum* had the lowest asymptotic length

and the highest growth constant. Thus, within these two species, a higher asymptotic growth length was coupled with having a lower growth constant.

Elevated growth rates at downstream sites may be driven by higher algal productivity and greater abundance of fine particulate organic matter typically found downstream (Vannote et al., 1980; Zieritz et al., 2010; Moss, 2011). It is also possible that faster sporadic flow rates in upstream locations of lowland rivers such as the Medway may impose further restrictions on shell morphology that constrain growth rates (Zieritz and Aldridge, 2009). Similar downstream growth patterns have been found from rivers elsewhere (e.g. mussels in the Colorado River Delta, Mexico and fish in the Warta River, Poland) and have been explained by increasing salinity, reduced oxygen uptake, increasing amounts of fine-grained sediments, and increasing food availability downstream (Ansell, 1968; Navarro, 1988; Przybylski, 1996; Schöne et al., 2003a).

4.2 Temperature effects on growth

Water temperature variation in the River Medway affected mussel growth in all four seasons during our study period. This relationship was positive in summer, spring, and winter, meaning mussel growth increased with rising water temperatures. Conversely, this relationship was negative in autumn, and mussel growth declined with rising water temperatures.

The increase in growth with temperature from winter to summer is most likely due to the fact that higher temperatures increase the metabolic activity of mussels and extend the annual growing period, therefore increasing the rate of shell production (Bolotov et al., 2018). Along with the direct physiological effects that water temperature has on mussel growth, there are many indirect effects of temperature which may also impact growth, reproduction, and survival.

Previous studies have shown strong correlations between temperature and various environmental conditions which drive mussel growth, many of which increase with rising temperatures; these include phytoplankton abundance (Robarts and Zohary, 1987), particulate organic carbon (POC) (Page and Hubbard, 1987; Kasai et al., 2010), chlorophyll *a* concentration (an indicator of phytoplankton levels) (Kautsky, 1982; Kasai et al., 2010; Kasai and Hirakawa, 2015; Tanabe et al., 2017), excretion of dissolved organic carbon (DOC) from phytoplankton (Zlotnik and Dubinsky, 1989) and nutrient availability (nitrogen, phosphorous, and silicate) (Rhee and Gotham, 1981; Kasai et al., 2010), all reaching peak levels around the same time as the main growing season of mussels.

While warmer temperatures generally resulted in positive growth, our study also documented a decline in growth at higher autumnal temperatures. Similar reduction in growth of unionid mussels during elevated temperatures has been reported by Mann and Glomb (1978), Mann (1979), and Bayne and Worrall (1980). The negative relationship between increasing autumnal temperatures and mussel growth may result from attempting to grow (driven by an increased metabolism) at a time of limited food quality and quantity, resulting in an overall net loss of energy and a decrease in the ability to put on weight or increase shell length. In addition to the stress of high temperatures, species *U. tumidus* and *U. pictorum* already have high metabolic demands during the autumn due to the energy requirement of tissue histolysis and histogenesis following their summer spawning period (Mann, 1979; Aldridge, 1999). Therefore, the temperature-induced additional metabolic drive during a season of re-growth and low food availability may hinder overall shell production.

It is important to note that an increase in growth rate does not necessarily indicate a healthier population. Although increases in nutrients and phytoplankton (following temperature rises) promote increased growth rates in mussels, these higher growth rates can result in reduced

longevity, abundance, and species richness (Haag and Rypel, 2011; Ridgway et al., 2011; Fritts et al., 2017). While this study demonstrates that mussel growth banding is significantly affected by seasonal water temperatures, and therefore documents valuable climate change data within freshwater ecosystems, understanding the full impact of temperature-accelerated growth rates on mussel communities and other freshwater biota requires further investigation. Additionally, other water parameters that often differ per location, such as hydrology, and biotic factors, such as population density and parasite load (Zieritz & Aldridge, 2011), were not measured in this study and may have had further effects on annual growth.

4.3 Freshwater mussels as indicators of climate patterns

Among our study species, annual summer water temperatures followed by spring water temperatures had the biggest effects on growth indices, supporting the knowledge that mussels grow mainly in the warmer months of spring and summer. The significant effects of seasonal temperatures on growth indices suggest that it is possible to use mussel growth increments to study past freshwater climate trends, particularly those of warmer seasons, and indicate that projected climate change will affect the future growth patterns of freshwater mussels.

By using growth rings to represent warmer or colder years, sclerochronology records can be created for the UK river systems to study long-term temperature fluctuations, which may help us to understand environmental changes occurring in these freshwater ecosystems. Long-lived, easily identifiable species at a single site may allow for long-term chronologies to be established, as is developed for marine bivalves. Since *U. tumidus* has the highest asymptotic lengths and takes the longest amount of time to reach these lengths, individuals of this species could contain the most rings and thus hold longer environmental records. Upon looking at individual species

trends, *U. tumidus* also shows the greatest separation in asymptotic length and growth constant trends across sites suggesting that it might be the best species for studying water parameters between locations. While annual mussel growth increments had significant relationships with seasonal climate data, the species in this study only provided between three to seven growth rings, limiting the amount of climate data we could match. It would be beneficial, therefore, to create chronologies from longer-lived species, such as *Anodonta cygnea* (Linnaeus) (the swan mussel) (Aldridge, 1999) to create more comprehensive freshwater histories. Additional use of museum collections with known collection dates would allow further cross-matching of *in situ* shell material which can last for decades with intact annuli (Bauer, 1992). Such studies may establish robust baselines against which future changes in growth rates can be compared, and thus may serve as an early warning of impending changes to mussel populations. This idea has already been utilised in dendrochronology; a study performed in Iran used tree-rings and an artificial neural network (ANN) to create a model that simulated groundwater levels from tree-ring diameter and precipitation inputs (Gholami et al., 2015). Given the ecosystem engineering role of freshwater mussels, changes in mussel size or population structure could impact entire freshwater communities. Documenting valuable information, their annual bands might allow us to determine how whole ecosystems are responding to environmental changes.

Species-level and habitat-level effects can make a difference on interpretations of growth when using mussels as indicators of temperature change. The annual pattern of mean standardised growth indices of *A. anatina* and *U. pictorum* were highly correlated within all three sites, implying that annual effects dominated site effects. However, while these annual growth trends were relatively consistent down the river, they often opposed the growth trends of *U. tumidus*. This further emphasizes the importance of species in the study of environmental parameters since they may differ in habitat preferences and respond differently to environmental

change. Between our three species, *A. anatina* prefers sandy and gravel substrate (Bauer and Wächtler, 2001), *U. pictorum* prefers firm, muddy substrate (Aldridge, 1999), and *U. tumidus* prefers finely grained clay substrate (Annie et al., 2013). Zajac et al. (2016) revealed that while *A. anatina* and *U. pictorum* occupied similar niches in a single lake, *U. tumidus* was the only mussel species of the five studied that showed no relation to any of the measured habitat variables. While all three of our study sites support diverse and abundant mussel communities, they differ in physical composition. Haysden consisted mainly of gravel sediment, Wateringbury was dominated by organic pellets and silt, and Ringlestone was characterised by a sticky clay substrate. Ringlestone contained the most favourable substrate for *U. tumidus*, which could explain why the greatest differences in *U. tumidus* growth compared to the other species occurred at this site (years 2010, 2011, 2014, and 2015). Since *U. tumidus* may have greater growth potential in Ringlestone (significantly higher L_{∞} at this site), drastic temperature changes might have a more noticeable effect on growth.

Different spawning and fertilisation times between species may also have an impact on their response to changing seasonal temperatures. Aldridge (1999) found mature glochidia in the outer demibranchs of both *Unio* species from May to June (summer), and of *A. anatina* from December to March (winter into spring). This could account for some of the varying trends in the standardised growth, with particularly slowed growth associated with periods when brooding coincides with especially unfavourable temperatures for growth. For example, in 2012, low summer temperatures correlated with a low growth index in *U. tumidus*, and in 2014, low spring temperatures correlated with a low growth index in *A. anatina*.

When using annuli of mussels as indicators to past climate trends, understanding the competing effects of seasonality as well as species-level and habitat-level effects can help us to build better chronologies based on the specific mussels being studied. Overall, using longer-lived

species from downstream populations could provide the strongest data on historical spring and summer temperatures in river systems. Eventually, by using established relationships between growth and temperature, we could cross-reference banding patterns on older, un-dated *in situ* shells to create longer temperature chronologies and reconstruct historical climates.

While seasonal temperatures have been shown to be significant correlates of growth in this study, it is possible that additional parameters may be important and some of these may be directly associated with temperature. Further studies might investigate the effects on growth of phytoplankton levels, POC, DOC, water flow rates, and nutrient levels such as N, P, and silicate. Future work could also include the effects of sex, additional locations, and more species (preferably longer living species). Patterns in additional river systems and over longer timeframes could also be considered. Ultimately, it may be possible to use mussel annuli patterns to reconstruct historical climatic patterns.

5. Conclusions

Freshwater ecosystems are highly vulnerable to climate change and are experiencing global habitat loss, species declines, and population shifts (Dudgeon et al., 2006; Markovic et al., 2014). Current biomonitoring schemes may be undermined by climate change since they are largely based on responses to organic pollution with little consideration of the increasing influence of climate (Woodward et al., 2010). Presently, we have relatively few tools available for understanding temperature patterns in freshwater systems, and those that exist often require destructive sampling and specialist equipment. Mussels offer an attractive tool for documenting water temperatures due to their clear annuli (capable of documenting large amounts of environmental information), longevity, global dispersal, accessibility, and the ability to measure

their growth non-destructively. The established relationship between winter, spring, and summer temperatures and annuli length will aid in the use of mussels to study past freshwater temperatures and to understand the impacts that climate change may have on freshwater ecosystems in the future.

Declarations of interest

None.

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