Validation of annual growth rings in freshwater mussel shells using cross dating

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Abstract: We examined the usefulness of dendrochronological cross-dating methods for studying long-term, interannual growth patterns in freshwater mussels, including validation of annual shell ring formation. Using 13 species from three rivers, we measured increment widths between putative annual rings on shell thin sections and then removed age-related variation by standardizing measurement time series using cubic splines. Initially, cross dating was a valuable quality control technique allowing us to correct interpretive and measurement errors in 16% of specimens. For all species, growth varied among years but was highly synchronous among individuals. Standardized measurement time series of 94% of individuals were significantly correlated with species master chronologies, and mean interseries correlations ranged from 0.37 to 0.96. Growth was also synchronous among species, even from different rivers, and growth was negatively correlated with mean annual streamflow for most species except *Quadrula pustulosa* from a regulated dam tailrace. Highly synchronous growth and the strong relationship to streamflow showed that large-scale environmental signals generated non-age-related variation in mussel growth giving strong support for annual formation of the growth increments we measured. Cross dating can be a valuable technique for studying freshwater mussel growth providing quality control, validation of annual rings, and reconstruction of long-term growth histories.

Résumé : Nous examinons l'utilité des méthodes dendrochronologiques de datation croisée dans l'étude des patrons interannuels de croissance à long terme chez les moules d'eau douce et, en particulier, pour la validation de la formation des anneaux annuels sur la coquille. Chez 13 espèces provenant de trois rivières, nous avons mesuré la largeur des incréments entre les anneaux annuels présumés sur des coupes minces de coquille, puis nous avons éliminé la variation due à l'âge en standardisant les séries chronologiques de mesures à l'aide de fonctions splines de degré 3. Au départ, la datation croisée s'est avérée être une technique précieuse de contrôle de la qualité qui nous a permis de corriger les erreurs d'interprétation et de mesure chez 16 % des spécimens. Chez toutes les espèces, la croissance varie d'une année à l'autre, mais est très synchronisée entre les individus. Les séries chronologiques standardisées des mesures de 94 % des individus sont en corrélation significative avec les chronologies principales des espèces et les corrélations moyennes entre les séries varient de 0,37 à 0,96. La croissance est aussi synchronisée entre les espèces, même de rivières différentes, et la croissance est en corrélation négative avec le débit moyen annuel pour la plupart des espèces, à l'exception de *Quadrula pustulosa* dans le canal de fuite d'un barrage de régulation des eaux. La croissance fortement synchronisée et la corrélation élevée avec le débit montrent que des signaux environnementaux à grande échelle causent une variation non reliée à l'âge dans la croissance des moules, ce qui confirme nettement la formation annuelle des incréments de croissance que nous avons mesurés. La datation croisée peut être une méthode précieuse pour étudier la croissance des moules d'eau douce en fournissant un contrôle de la qualité, en validant les anneaux annuels et en permettant la reconstruction du déroulement de la croissance sur une grande échelle temporelle.

[Traduit par la Rédaction]

Introduction

Annual growth increments are often prominent features of bivalve shells and can provide a wealth of information about age and growth of these animals. Validation of the annual formation of growth increments is an essential prerequisite for deriving age and growth information from shell annual rings or analogous structures in any organism (Beamish and McFarlane 1983). In addition to confirming timing of growth increment formation, validation studies decrease errors in interpretation of these features (DeVries and Frie 1996; Campana 2001; Haag and Commens-Carson 2008). Although annual formation of growth increments has been demonstrated for many marine bivalves, few validation studies have been conducted for freshwater mussels (see Haag and Commens-Carson 2008). To utilize the full potential of growth increments as indicators of mussel age and growth, annual formation of shell rings needs to be validated for a large number of species across different habitats and regions using robust, repeatable methods.

Most validation studies for freshwater mussels have used mark–recapture methods involving marking shells, returning marked mussels to their habitat, and then retrieving them at least one year later to examine shell growth deposited after

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marking (e.g., Neves and Moyer 1988; Howard and Cuffey 2006; Haag and Commens-Carson 2008). This approach can be effective but is time consuming (requiring at least one full year) and often suffers from a low return of marked animals in dynamic stream habitats. Further, handling of study specimens causes deposition of disturbance rings that are superficially similar to annual rings and can result in reduced growth, both potentially leading to erroneous conclusions about the periodicity of growth rings (Haag and Commens-Carson 2008). The disadvantages of mark–recapture techniques for freshwater mussels highlight a need for alternative and less intrusive methods of independently validating putative shell annual rings.

Cross dating is a fundamental technique of dendrochronology by which high frequency patterns of annual variation in tree ring width (reflecting variation in growth) are used to validate the timing of ring formation and to ensure that all increments have been correctly identified and assigned the correct calendar year (Douglas 1939). Because growth increments in trees are influenced by climate, long-term growth chronologies of individual trees can be highly synchronous within a population and the mean chronology of a population (referred to as the master chronology) is often correlated with annual variations in climate. Therefore, interpretation of annual ring formation for an individual tree can be validated if the standardized measurement time series for that individual is correlated with the master chronology for the population; this is referred to as the sample–master correlation (Grissino-Mayer 2001; Esper et al. 2002). Further, aligning and cross matching each standardized measurement time series with the master chronology can reveal growth anomalies or errors in ring interpretation and can allow archeological dating of wood specimens of unknown age provided a master chronology for that species already exists (Douglas 1921; Alestalo 1971; Grissino-Mayer 2001).

Dendrochronological techniques have been applied to other taxa such as freshwater and marine fishes, corals, and marine mollusks forming the basis of an emerging science known as “sclerochronology” (Pereira et al. 1995; Strom et al. 2004; Helama et al. 2006). Because growth of poikilothermic aquatic animals is also directly influenced by climate, as well as by hydrologic conditions, sclerochronological cross dating has been applied to validate and study annual growth increments in fish otoliths and fin rays, marine bivalve shells, and shells of the Holarctic pearl mussel *Margaritifera margaritifera* (Pereira et al. 1995; Black et al. 2005; Helama et al. 2006).

We apply cross dating to evaluate the assumption of annual deposition of shell rings in 13 freshwater mussel species from three rivers in the southeastern United States. First, we examine patterns of shell ring formation and assess whether observed levels of variation are useful for cross dating. Second, we examine synchronicity of growth among individuals in a population and use the resulting interseries correlations to validate annual rings and identify growth anomalies or potential errors in interpretation of shell thin sections. Third, we compare master chronologies of different species within and among rivers to explore the generality of observed patterns of variation. Fourth, we examine relationships between annual variation in mussel growth and climate and hydrological variables. Finally, we discuss how cross dating can be used either with mark–recapture methods or as a stand-alone method for validating the assumption of annual formation of shell rings in freshwater mussels.

Materials and methods

Study sites and species

Mussels were collected from one site in the Little Tallahatchie River, Panola County, Mississippi (34°23′56″N, 89°47′33″W), one site in the St. Francis River, Cross County, Arkansas (35°16′12″N, 90°34′58″W), and at two sites in the Sipsey River, Pickens–Greene Counties, Alabama (33°07′16″N, 87°55′08″W; 33°05′16″N, 87°57′27″W). All four sites support diverse and abundant mussel communities, but physical habitat conditions differ greatly among the streams. The Little Tallahatchie River is regulated and impounded; our site was located in the dam tailrace below a major storage reservoir (Sardis Reservoir) and is impounded by a low-head dam about 2–3 km downstream of the site (Haag and Warren 2007). Substrate at the Little Tallahatchie River site was composed primarily of sand, and water depth was approximately 3.0 m. The St. Francis River is largely unregulated in the vicinity of our study site, but much of the watershed is affected by channelization and water diversion projects; land use in the watershed is dominated by large-scale, intensive agriculture and the river receives heavy runoff from these activities (Ahlstedt and Jenkinson 1991). Substrate at the St. Francis River site was mostly silt with some sand, and water depth was 1.0–1.5 m. The Sipsey River is unregulated and unmodified and the watershed is mostly forested; consequently, water quality is high and the river supports one of the most intact aquatic communities in the region (McCullagh et al. 2002). The Sipsey River sites were within 10 km of each other and had stable gravel and sand substrates and water depths from ~0.1 to 1.2 m. At all sites, we collected mussels from within about 100–200 m segments of stream channel.

Thirteen mussel species were used in this study (Table 1; total shells examined = 203), and with the exception of *Elliptio crassidens* and *Fusconaia ebena*, all 13 study species were common constituents of mussel communities at the study sites (Table 1). We included *E. crassidens* and *F. ebena* because the high number of rings present in our specimens allowed construction of lengthy growth chronologies. Study species represented a diversity of life history traits, including host fish use (Haag and Warren 2003), fecundity (Haag and Staton 2003), and longevity. Most study species grow relatively slowly and live to >30 years. However, *Lampsilis ornata*, *Lampsilis teres*, and *Potamilus purpuratus* grow rapidly and rarely exceed 20 years of age (W.R. Haag, unpublished data). Growth and longevity of *Quadrula quadrula* and *Quadrula verrucosa* are intermediate; both of these species commonly live >20 years, but we had no older specimens in this study. The assumption of annual ring formation has been validated previously at these same study sites using mark–recapture methods for all species in the present study except *E. crassidens*, *F. ebena*, *Obovaria unicolor*, and *Pleurobema decisum* (Haag and Commens-Carson 2008).

Table 1. Cross-dating statistics for 13 mussel species from southeastern USA.

				% of individuals validated		Mean interseries *R* (Pearson's)	
Species	Time series	*N*	Optimal spline flexibility	Before QC	After QC	Before QC	After QC
Sipsey River							
Elliptio arca	1974–2005	21	24	76	95	0.39	0.50
Elliptio crassidens	1949–1999	10	40	100	100	0.50	0.50
Fusconaia cerina	1954–2005	29	24	76	93	0.25	0.49
Fusconaia. ebena	1955–1999	5	16	80	80	0.37	0.37
Lampsilis ornata	1982–2005	23	24	87	91	0.48	0.60
Obovaria unicolor	1963–1998	8	8	88	88	0.37	0.55
Pleurobema decisum	1955–1999	12	16	50	92	0.20	0.37
Quadrula asperata	1966–2005	26	32	77	96	0.46	0.49
Quadrula (*Tritogonia*) *verrucosa*	1982–1999	17	32	82	100	0.42	0.56
St. Francis River							
Lampsilis teres	1993–2002	9	16	67	100	0.44	0.76
Potamilus purpuratus	1995–2003	8	8	75	100	0.44	0.96
Quadrula quadrula	1991–2003	5	24	80	100	0.36	0.49
Little Tallahatchie River							
Quadrula pustulosa	1966–2003	17	32	65	77	0.38	0.52

Note: *N*, number of shells examined for each population; optimal spline flexibility, the value that resulted in the highest mean interseries correlation for that species; % validated, the percentage of individuals with measurement time series that were significantly and positively correlated with the master chronology for that species; QC, quality control.

Collection and preparation of shells

We collected live mussels, when possible, for all species except *P. decisum* by snorkeling and using SCUBA from 1996 to 2006. *Pleurobema decisum* is listed as endangered under the US Endangered Species Act and no live individuals were collected; we collected only freshly dead shells. In addition, we used freshly dead shells of other species if we were unable to collect sufficient numbers of live animals.

In the laboratory, we prepared radial thin sections (~300 µm thick) from one valve of each specimen using a low-speed saw with a diamond-impregnated blade (Buehler Ltd., Lake Bluff, Illinois). Our thin-section preparation methods are described in detail in Haag and Commens-Carson (2008) and are based on standard methods for bivalves (e.g., Clark 1980; Neves and Moyer 1988; Veinott and Cornett 1996).

Interpretation and measurement of shell rings

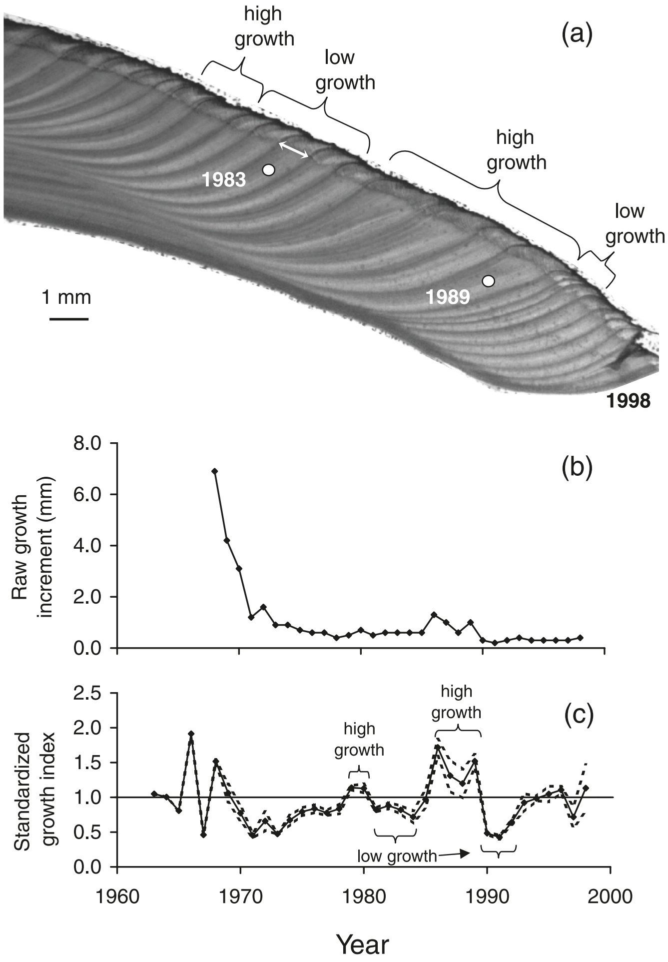
All thin sections were interpreted independently by two experienced observers using a binocular microscope. We identified annual rings and differentiated them from non-annual shell rings (e.g., disturbance rings) following criteria described by Haag and Commens-Carson (2008). Briefly, annual rings were continuous from the shell exterior to the umbonal region and were not associated with a disruption of the continuity of the prismatic shell layer as seen frequently in disturbance rings. In many cases, annual rings curved abruptly within the prismatic layer and were surrounded by a clear halo within this region of the shell. If the two observers differed in their interpretation of an increment and could not reach consensus following re-examination of the specimen, the specimen was excluded from further analysis.

We measured annual growth increments using a binocular microscope and digital camera interfaced with video-imaging software. For each specimen, we initiated measurements at the most recent complete growth increment (i.e., the year prior to collection) and worked backwards in time, allowing us to associate a specific calendar year with each growth increment. We measured growth increments as the linear dorsoventral distance (to the nearest 0.1 mm) between adjacent annual rings at the interface between the prismatic and nacreous shell layers (Fig. 1*a*); we used this measurement point because it offered a precise and unambiguous landmark that was usually unaffected by erosion of the periostracum or other external shell damage. Increments measured linearly may slightly underestimate growth in some individuals relative to curvilinear measurements made along the shell surface but the degree of curvature between increments is usually very small and the difference between these two measurements is negligible. We measured growth increments of three individuals of *F. ebena*, *Quadrula asperata*, and *L. ornata* (representing species of slow, intermediate, and fast growth, respectively), using both linear and curvilinear techniques. Correlation coefficients between the resulting standardized growth indices (see ARSTAN methods below) for the two measurement techniques were ≥0.95 for all species. Shell erosion in the umbonal region of many specimens precluded creation of a complete growth series, but in general, growth increments were incomplete or absent for no more than five of a specimen's earliest years.

Cross dating and analysis of shell ring patterns

We cross-dated each standardized measurement time series within a species using the dendrochronology program, COFECHA (http://web.utk.edu/~grissino/software.htm; Holmes 1983; Grissino-Mayer 2001). We generated standardized index series by removing age-related variation in growth using a flexible cubic spline (Cook and Peters

Fig. 1. Example of master chronologies for *Obovaria unicolor* from the Sipsey River, Alabama. (*a*) Thin section of specimen showing annual variation in growth increment width including alternating periods of high and low growth. From left to right, periods represent 2, 4, 4, and 3 years of growth, respectively. Years are indicated on the cross section for reference purposes. White arrow in 1983 shows measurement dimension for growth increments. (*b*) Raw measurement time series for specimen figured above showing strong age-related variation in growth. (*c*) Master chronology for *Obovaria unicolor* (n = 8 individuals) showing annual variation in growth unrelated to age. y axis is a unitless index of growth with mean = 1. Indicated high and low growth periods correspond to those in panel *a*. Standard errors for each year are indicated by broken lines.



1981). For each species, we determined optimal spline flexibility by applying a range of flexibilities and choosing the value that resulted in the highest mean interseries correlation (R) within the master chronology (Black et al. 2005; Helama et al. 2006; see below and Table 1). For each species, we generated a master growth chronology by computing the average standardized growth increment for each calendar year of record. We then correlated standardized measurement time series with an adjusted master chronology that was created by omitting the series of that particular individual (Grissino-Mayer 2001). Individuals that were significantly ($\alpha < 0.05$) and positively correlated with the master chronology were considered validated and the mean interseries correlation coefficient across all individuals was used to assess the overall robustness of the annual growth pattern for each species.

We examined each specimen for potential interpretive errors by lagging each standardized measurement time series forward and backward by 1–3 years and correlating the lagged series with the master chronology (Grissino-Mayer 2001). If the correlation coefficient was strongest with the series in its original placement in time, we considered that the shell thin section had been interpreted correctly (Holmes 1983). If correlation coefficients were stronger after lagging, we re-examined the specimen for potential errors in interpretation. If interpretive errors were found after re-examination (e.g., missed annual rings, nonannual rings counted as annual), we remeasured increment widths for the entire specimen and constructed a corrected standardized measurement time series (Black et al. 2005). If no obvious interpretative errors were found, we retained the specimen in the data set with its original measurements. After this quality control technique was completed, we repeated the entire cross-dating procedure using the corrected data set.

To illustrate this cross-dating quality control procedure, we simulated an error for one *Fusconaia cerina* individual by combining the 1979 and 1980 growth increments for this shell, thus displacing the series by –1 years. This dropped the individual's correlation coefficient with the adjusted master chronology from 0.54 to 0.15. When this individual was run through the COFECHA cross-dating routine, the program accurately detected this error and suggested that by lagging the series by –1 years the correlation coefficient could be improved to 0.42.

Following cross dating and quality control, we transformed corrected, raw growth increment data into growth indices using another dendrochronology software package, ARSTAN (http://www.ldeo.columbia.edu/res/fac/trl/public/publicSoftware.html; Cook and Holmes 1984; for a discussion of ecological applications of ARSTAN and COFECHA, see Drake and Naiman 2007). Similar to COFECHA, ARSTAN removes age-related growth trends by using a detrending function, but users specify one of a number of detrending algorithmic options. To retain as much of the low-frequency climatic signature as possible, "stiffer" (e.g., negative linear and negative exponential) functions are often used at this stage (Fritts 1966). We therefore standardized each individual using a simple negative exponential curve and divided the actual increment value by that predicted from the curve. Detrending and removal of autocorrelation resulted in a unitless, standardized index of growth for each year with an average of 1; values above 1 represent above-average growth and values below 1 represent below-average growth. Using these growth indices we cross-correlated master growth chronologies of all species to examine similarities in growth patterns among species. For each species, we correlated the annual mean growth index with mean annual air temperature and mean annual streamflow (Pearson's product moment analysis; JMP statistical software, version 5.1, SAS Institute, Cary, North Carolina). We used data from the nearest available weather station or streamflow gage as follows: Little Tallahatchie River, Sardis Dam weather station, 1949–2004, Sardis Dam discharge data, 1947–2005 (US Army Corps of Engineers, Sardis Lake

Field Office, Sardis, Mississippi); St. Francis River, Memphis International Airport weather station, 1940–2006, US Geological Survey streamflow gage 07047800 at Parkin, Arkansas, 1931–2006; Sipsey River, Tuscaloosa County Airport weather station, 1949–2006, US Geological Survey streamflow gage 02446500 at Elrod, Alabama, 1929–2006.

Results

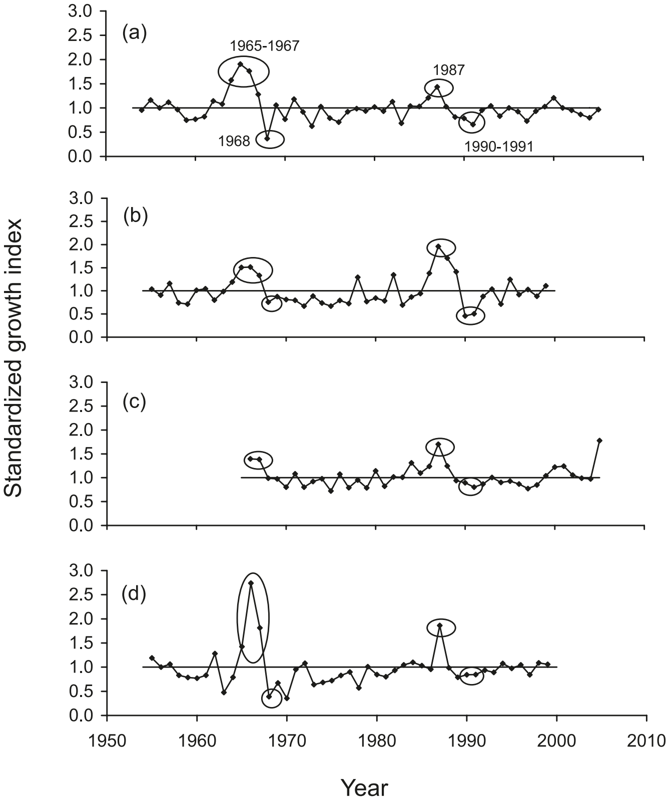
Master growth chronologies for each species ranged from 9 to 52 years (Table 1). Alternating periods of high and low growth were clearly visible on thin sections (Fig. 1*a*), and these patterns were borne out in the master growth chronologies (Figs. 1*b* and 1*c*). In addition, pointer years of exceptionally high or low growth were evident in many standardized measurement time series and were often similar among species (Fig. 2, note years 1965–1967, 1968, and 1987). The variation around the standardized growth index (mean = 1) was of similar magnitude for all species (coefficients of variation = 19.7%–39.4%).

Cross dating identified 49 specimens as potentially having interpretive or measurement errors. After re-examination of these specimens, we identified and corrected errors in 33 specimens (16% of total specimens examined in study). Three individuals were discarded because of extensive shell erosion that prevented a conclusive reassessment of rings, and we retained unchanged the measurement time series for 13 individuals for which we could identify no errors. Corrected errors were a result of both faulty interpretation of thin sections (e.g., inaccurate identification of annual shell rings) and errors in measurement of growth increments. Errors in the number of annual rings identified for an individual ranged from –5 (e.g., five nonannual rings initially identified as annual) to +2 (e.g., two annual rings missed). However, most errors involved a discrepancy of only one or two rings (the mean of absolute values of all corrected discrepancies = 1.6 rings). Correcting these errors increased the number of individuals validated and the strength of interseries correlations for most species (Table 1; see below).

Long-term patterns of mussel growth were highly synchronous among individuals within all species. After correcting for errors identified by cross dating, 94% of total individuals examined were considered validated (standardized measurement time series significantly correlated with the species' master chronology; Table 1). Greater than 90% of individuals were validated for all species except *O. unicolor* (87% validated) and *Quadrula pustulosa* (77% validated). Mean interseries correlations after quality control ranged from 0.37 to 0.96, showing that most individuals in a population share a high percentage of non-age-related variation in growth. The three fastest-growing species had the highest mean interseries correlations: *L. ornata*, *L. teres*, and *P. purpuratus* (0.60–0.96). Otherwise, interseries correlations for the majority of species were similar and were near 0.50 (average across all species = 0.55; Table 1).

Growth was also similar among many species (Table 2). At the Sipsey River, master growth chronologies of all species were significantly and positively correlated with at least two other species in the assemblage. *Elliptio crassidens*, *F. cerina*, and *F. ebena* were each correlated with all but one of the other species and *E. arca* and *Q. asperata* were correlated with all but two species. Master growth chronologies of all species in the St. Francis River were strongly and positively correlated with each other. Growth of many species was also similar among the Sipsey and St. Francis rivers. Growth of *Q. pustulosa* in the tailrace of Sardis Dam on the Little Tallahatchie River was correlated with only one species (*E. arca*, Sipsey River) but the correlation coefficient indicated a negative relationship; this was the only significant negative correlation among any pair of species.

Fig. 2. Non-age-related variation in annual growth of four species of from the Sipsey River, Alabama: (*a*) *Fusconaia cerina* (*n* = 29); (*b*) *Fusconaia ebena* (*n* = 5); (*c*) *Quadrula asperata* (*n* = 26); and (*d*) *Pleurobema decisum* (*n* = 12). Data are standardized master chronologies for each species. Ellipses (identified in *a*) highlight pointer years of exceptionally high or low growth that are shared by at least three species. Horizontal lines represent average annual growth for the population (growth index = 1). Standard errors were similar to those found in Fig. 1 and are excluded for graph clarity.



Growth of nearly all species was significantly negatively correlated with mean annual streamflow (Table 2). Growth of *P. decisum* and *Q. verrucosa* was also negatively correlated with streamflow but these correlations were not significant (*P* = 0.19 and 0.11, respectively). *Quadrula pustulosa*, collected from the tailrace of Sardis Reservoir, was the only species that did not show a relationship between streamflow and growth. No significant correlations between annual growth and annual air temperature were detected.

Discussion

Cross dating is a powerful tool for studying growth of freshwater mussels. Fundamental prerequisites for cross dat-

Table 2. Pearson correlations (with significant relationships ($\alpha < 0.05$) indicated in bold) between master chronologies of 13 southeastern USA mussel species (species abbreviations follow their names), mean annual streamflow, and mean annual air temperature.

Species	EA	EC	FC	FE	LO	OU	PD	QA	QV	LT	PP	QQ	QP
Sipsey River													
Elliptio arca (EA)	**1.00**												
Elliptio crassidens (EC)	**0.63**	**1.00**											
Fusconaia cerina (FC)	**0.48**	**0.53**	**1.00**										
Fusconaia ebena (FE)	**0.73**	**0.74**	**0.62**	**1.00**									
Lampsilis ornata (LO)	**0.47**	**0.50**	**0.44**	0.26	**1.00**								
Obovaria unicolor (OU)	**0.55**	**0.57**	0.25	**0.51**	**0.45**	**1.00**							
Pleurobema decisum (PD)	0.35	**0.47**	**0.67**	**0.49**	0.03	0.31	**1.00**						
Quadrula asperata (QA)	**0.42**	**0.72**	**0.58**	**0.71**	0.23	**0.37**	**0.65**	**1.00**					
Quadrula (*Tritogonia*) *verrucosa* (QV)	0.32	0.28	**0.48**	**0.39**	0.31	0.34	0.36	0.37	**1.00**				
St. Francis River													
Lampsilis teres (LT)	**0.69**	**0.76**	**0.72**	0.03	0.53	0.16	0.02	**0.88**	–0.05	**1.00**			
Potamilus purpuratus (PP)	0.46	0.04	**0.76**	0.37	0.49	0.26	0.37	**0.84**	0.64	**0.70**	**1.00**		
Quadrula quadrula (QQ)	**0.60**	0.64	0.52	0.02	**0.48**	0.03	–0.13	**0.75**	–0.33	**0.88**	**0.74**	**1.00**	
Little Tallahatchie River													
Quadrula pustulosa (QP)	**-0.38**	0.02	–0.14	–0.15	–0.16	0.19	–0.14	0.06	–0.38	0.18	0.32	0.39	**1.00**
Abiotic factors													
Mean annual streamflow	**-0.59**	**-0.42**	**-0.41**	**-0.51**	**-0.46**	**-0.38**	–0.23	**-0.35**	–0.40	**-0.60**	**-0.93**	**-0.73**	0.10
Mean annual air temperature	0.08	–0.07	0.09	0.00	–0.14	–0.14	0.06	0.03	0.27	–0.23	0.09	–0.27	–0.03

ing are as follows: (*i*) growth shows substantial non-age-related variation and (*ii*) growth is synchronous among individuals within a population (Black et al. 2005; Helama et al. 2006); the growth patterns that we observed for freshwater mussels satisfy these conditions. After removing age-related variation, growth of all species showed years of high and low growth interspersed among average growth years. Significant mean interseries correlations for all species showed that a high percentage of non-age-related growth variation was shared among individuals within a population. The level of growth synchrony that we observed was similar to or higher than that reported in cross-dating studies for a wide range of other organisms, including marine and freshwater bivalves (Table 3).

Although based on standard and well-defined dendrochronology techniques, cross-dating methods for freshwater mussels are not well established. For example, we found it necessary to iteratively determine optimal spline flexibilities used in our detrending algorithms and these splines varied among mussel species, in contrast to trees which have splines that are often optimized at a flexibility of about 32 years (Grissino-Mayer 2001). However, our sclerochronologies (and many other sclerochronologies) are shorter than most tree-ring chronologies, which are rarely less than 100 years long. Detrending functions differed among mussels likely because of their short chronologies and variability in life span of study species. These results are similar to previous sclerochronological studies with other animals that required case-by-case development of optimal detrending functions (e.g., Helama et al. 2006), highlighting the need for experimental optimization when cross-dating taxa for which little previous guidance exists.

In addition to interpretation of statistical results, such as those provided by COFECHA, validation of shell rings should always be accompanied by a visual or graphical assessment of growth patterns. A combination of visual and mathematical techniques will undoubtedly provide the best cross-dating results. For some individuals, visual cues alone might be sufficient to successfully cross-date a sample even without measurement of growth increments and subsequent statistical analysis (Yamaguchi 1991). In more subtle cases, increment measurement and chronology creation can provide effective cross dating not immediately apparent to the naked eye. However, identification of pointer years, both visually and graphically (here, years where growth index is $>|0.5|$), is critical for cross dating as they provide natural markers that can be used to validate growth increments and demonstrate growth synchrony. Pointer years also provide information about effects of extreme environmental events on growth and can be used to align measurement series from different individuals, allowing placement in absolute time of specimens with otherwise unknown temporal context (e.g., tree stumps, long-dead mussel shells) (Douglas 1921; Fritts 1971; Helama et al. 2007). In our study, several consistent pointer years were evident, providing reference points for qualitatively checking the alignment of chronologies. Other visual cues potentially useful for cross-dating mussels include disturbance rings and diffuse dark bands. In this study, we do not examine the utility of these markers for cross dating. However, because they occur commonly in mussels, disturbance rings and other visual cues need to be studied more thoroughly to assess their potential as markers for cross dating. Even if natural events sufficient to produce disturbance marks in a high percentage of individuals are rare, less severe events may be recorded in other ways. A mass appearance of diffuse dark bands was observed in 2004 in several mussel species in the Little Tallahatchie River, Mississippi, but not in other years or other rivers (Haag and Commens-Carson 2008). These visual markers along with statistical approaches to cross dating (e.g., those provided by COFECHA) offer an array of tools with which to cross-date and validate rings in freshwater mussel shells.

Cross dating and subsequent analysis of shell rings can be used to validate the assumption of annual deposition of shell

Table 3. Comparison of interseries correlation coefficients using tree-ring cross-dating techniques for a variety of taxa and ecosystems globally.

Species	Location	Mean or range of interseries *R*	Source
Lake sturgeon (*Acipenser fulvescens*)	Canadian Rivers	0.11	LeBreton et al. 1999
Splitnose rockfish (*Sebastes diploproa*)	Pacific Ocean	0.53	Black et al. 2005
Scots pine (*Pinus sylvestris*)	Scandinavia	0.21–0.58	Helama et al. 2007
Baldcypress (*Taxodium distichum*)	Southeastern USA	0.48	Stahle et al. 1985
White oak (*Quercus alba*)	Quebec	0.42–0.66	Tardif et al. 2006
Red oak (*Quercus rubra*)	Quebec	0.38–0.66	Tardif et al. 2006
Holarctic pearl mussel (*Margaritifera margaritifera*)	Scandinavia	0.39–0.67	Helama et al. 2006
Ocean quahog (*Arctica islandica*)	Scandinavia	0.48	Helama et al. 2007
Southeastern USA mussels	Southeastern USA	0.37–0.96	This study

rings in freshwater mussels. Using cross dating, we showed conclusively that shell growth increments are formed in synchrony among individuals and species and, therefore, that regular formation of growth rings is a pervasive feature of mussel growth. However, synchrony of growth ring formation alone is not necessarily indicative of annual formation of these rings. For all but four of our study species (see next sentence), the annual formation of growth rings has been demonstrated previously and independently using a mark–recapture approach (Haag and Commens-Carson 2008), confirming that the synchronous growth patterns we observed are indeed reflective of annual phenomena. Annual formation of growth rings by *E. crassidens*, *F. ebena*, *O. unicolor*, or *P. decisum* has not been previously validated but is supported by the strong correlation of their master chronologies with the master chronologies of species demonstrated to produce annual rings through mark–recapture. Even without independent confirmation of annual ring formation from mark–recapture studies, the significant correlations of master growth chronologies with annual variation in streamflow for all species except *Q. pustulosa* provide strong evidence that these chronologies and the rings on which they are based reflect annual patterns of growth.

One of the greatest challenges in interpreting mussel shell rings, even after validation, is differentiating annual rings from nonannual rings, particularly those caused by disturbance. Although these rings often have consistent, diagnostic characteristics (Neves and Moyer 1988; Haag and Commens-Carson 2008), differentiation can be challenging, particularly when disturbance rings are superimposed over or located adjacent to annual rings, masking or conflating two independent events in the individual's growth history. Cross dating and subsequent lagging of time series was an effective quality control technique and identified errors in both interpretation of thin sections (e.g., differentiating between annual and nonannual shell rings) and measurement of growth increments. At least 16% of our specimens had some form of interpretive or measurement error, showing that even independent analysis by two experienced readers can result in errors. For this reason, we encourage routine application of cross dating to studies involving mussel shell ring interpretation.

A small percentage of specimens in our study remained unvalidated after quality control. This is common in dendrochronology and can be interpreted as being caused by variable degrees of stress, disturbance, or injury experienced by individuals (Grissino-Mayer 2001). Freshwater mussels likely also experience growth anomalies or trauma that result in deviation from growth synchrony (e.g., predation attempts, flood displacements). Alternatively, specimens could have retained interpretive errors that we were unable to detect even after re-examination. Interpretive errors are a common risk in all growth studies but cross dating minimizes their impact; the agreement of our interseries correlations with a wide variety of other cross-dating studies confirmed the overall robustness and accuracy of our interpretations.

The strong correlations of growth among species within a community, among species in different rivers, and with mean annual streamflow suggest that mussel growth is influenced by pervasive environmental factors. Mussel growth was influenced similarly by streamflow, and potentially other factors, at a regional scale, as the Sipsey River and St. Francis River sites are separated by over 350 km. In contrast, growth of *Q. pustulosa* in the Little Tallahatchie River was not correlated with streamflow or with other species, likely because the dam tailrace habitat is uncoupled from natural variations in streamflow (e.g., Poff et al. 1997). Growth of all other species showed significant or near significant negative correlations, with streamflow indicating that high flow years produce lower growth relative to low flow years. In a previous study, mussel survival was lower in years of extremely high flows (Villella et al. 2004), but virtually nothing is known about the effect of streamflow on freshwater mussel growth. Our findings suggest that streamflow is an important factor in mussel growth; cross dating, followed by analysis of shell ring patterns, will allow investigation of this understudied relationship. Growth of *Margaritifera margaritifera* in Sweden was strongly correlated with summer air temperature, but the strength of this relationship decreased from north to south (Schöne et al. 2004), suggesting that mussels at higher latitudes are limited by availability of warm temperatures favorable for growth. The lack of correlation between temperature and growth for any species in our study suggests that temperature is not a limiting factor for growth in warmwater temperate rivers.

In most organisms, production of distinct growth rings is associated with a strong, predictable annual cycle in some environmental variable(s) (Black et al. 2005; Tardif et al. 2006). Growth rings in temperate-zone trees, fishes, and marine bivalves are produced during periods of growth cessation in winter (LeBreton and Beamish 2000; Schöne et al. 2002; Rypel et al. 2006), whereas growth rings in tropical treeline trees are produced in response to slow growth in the dry season (Biondi 2001). Distinct nonannual tree rings (e.g., disturbance rings) can occur in all individuals in a population in response to some major environmental event (e.g., fire, drought), but such rings are sporadic and irregular in occurrence (Payette et al. 1990; Barber et al. 2000; Kulakowski and Veblen 2007). Regularly occurring intraannual rings such as spawning checks in fishes or bivalves are usually readily distinguishable from more distinct rings formed at the end of the growing season (Jones 1980; Campana 2001). For these reasons, formation of distinct annual growth increments is a nearly universally held tenet for many organisms (Campana and Thorrold 2001) and validation of this assumption is often accomplished and accepted on the basis of cross dating alone (Black et al. 2005). A growing body of evidence supports the production of annual winter rings in a taxonomically wide variety of freshwater mussels from a wide variety of habitats (e.g., Mutvei and Westermark 2001; Valdovinos and Pedreros 2007; Haag and Commens-Carson 2008), opening the possibility that cross dating can be used as a stand-alone technique for validation of shell rings.

Widespread validation of annual shell rings in mussels has not taken place, thus the strongest validation approach remains utilization of a mark–recapture study with cross dating to provide independent evidence for annual periodicity and identification of interpretive errors. However, the requirement of an independent mark–recapture study can be obviated in some cases. First, if annual ring formation has been validated in other species in an assemblage or region, a high correlation between master chronologies of these species with unstudied species provides strong evidence for annual ring formation in the unstudied species. Second, even if no information exists for other species in an area, a high correlation between putative annual growth increments and a strong, variable environmental signal (e.g., annual streamflow) can provide convincing support for the annual formation of shell rings. We used cross dating to show that freshwater mussels produce annual rings, individuals within and among populations show synchronicity of growth, and mussel growth is negatively correlated with mean annual streamflow. Although cross dating has rarely been applied to studies of freshwater mussel growth, its use will help unlock the vast information contained within shell rings.

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