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Research paper

Three approaches to radiocarbon calibration of amino acid racemization in *Mulinia lateralis* from the Holocene of the Chesapeake Bay, USA

Amy E. Simonson [a,1], Rowan Lockwood [b,*], John F. Wehmiller [a]

[a] Department of Geological Sciences, College of Earth, Ocean, and Environment (CEOE), University of Delaware, Newark, DE 19716, USA
[b] Department of Geology, The College of William and Mary, P.O. Box 8795, Williamsburg, VA 23187, USA

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ABSTRACT

A radiocarbon-calibrated aminochronology, based on the bivalve *Mulinia lateralis*, is presented for Chesapeake Bay core MD03-2661, a 25 m piston core drilled near Kent Island (38°53.21′N; 76°23.89′W) during the 2003 USGS Marion-Dufresne cruise. Three separate approaches were used to calibrate amino acid racemization (AAR) data for aspartic acid with radiocarbon data. For the first approach, a direct or paired analysis calibration incorporated eight articulated specimens, thereby allowing for the application of AAR and radiocarbon analysis of the same specimen and effectively eliminating both intrashell variability and time averaging as factors in the calibration. A second direct approach relied on valves that were bilaterally split to facilitate both AAR and radiocarbon dating, thus effectively eliminating time averaging effects from this calibration. For the third indirect approach, nine independent radiocarbon dates were combined with 129 Asx D/L ratios from the same core depths to produce an indirect calibration model, from which intershell variability and time averaging could be estimated. Variability in AAR ratios was recognized from a myriad of sources, including analytical error, intrashell variability, inherent variability, time averaging, and contamination. The majority of this variability was controlled for through experimental design or by the application of these three independent calibration approaches. The direct calibration of articulated shells and the indirect calibration yielded virtually identical age models, well within their respective 95% confidence intervals. This study establishes an aminostratigraphic reference section for the Holocene record of the Chesapeake Bay and demonstrates the usefulness of multiple calibration approaches and the potential utility of AAR for future studies of sedimentary processes and chronologies in the bay.

1. Introduction

The Chesapeake Bay is the largest and most productive estuary in the U.S., but recent problems with water quality, including nutrient pollution and oxygen depletion, have led to an increased interest in establishing a timeline of environmental degradation (Karlsen et al., 2000; Brush, 2001; Jackson et al., 2001). The bay preserves a thick Holocene record due to sea level rise and subsequent infilling of the paleo-Susquehanna river valley, beginning approximately 8.0–7.6 ka (Colman and Mixon, 1988; Dowsett and Cronin, 1989; Bratton et al., 2003; Hobbs, 2004). Radiocarbon is the dating technique most commonly applied to Holocene material from the Chesapeake Bay (Willard et al., 2005; Cronin et al., 2007) but is costly when numerous samples are dated, hence other geochronology methods are needed.

Amino acid racemization (AAR) dating offers the potential to obtain high-resolution ages for samples less than 1000 years old (Goodfriend, 1989), but has yet to be applied to chronological problems in Chesapeake Bay. The present study is the first to establish a ^{14}C-AAR calibration curve for the Holocene strata of Chesapeake Bay, thereby contributing another geochronologic tool that may allow further understanding of Holocene sedimentation rates and potential time averaging. This study is novel in four other respects: (1) it is the first study to apply and compare three independent calibration techniques, (2) the comparison of these techniques yields estimates of sources of variability including age mixing, (3) the study applies the resulting age model to another core from a different location in the Chesapeake Bay and (4) it is the first to develop an AAR age model for a Holocene section in the Atlantic Coastal Plain with multiple depths in a single core.

* Corresponding author. Tel.: +1 757 221 2878; fax: +1 757 221 2093.
E-mail addresses: asimonso@usgs.gov (A.E. Simonson), rxlock@wm.edu (R. Lockwood), jwehm@UDel.Edu (J.F. Wehmiller).
[1] Present address: USGS New York Water Science Center, 2045 Route 112, Coram, NY 11727, USA.

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2. Study system

2.1. Chesapeake Bay

The Chesapeake Bay (Fig. 1) covers an area of approximately 11,400 km^2 and drains a 165,800 km^2 watershed (Larsen, 1998; Cronin et al., 1999). The bay preserves a thick Holocene sedimentary record with sedimentation rates of 1–5 mm per year (Colman et al., 2002). Severe water quality degradation in recent decades has spurred the need to assess the environmental conditions before European settlement in the late 1700s, in order to create reasonable restoration goals. It is estimated that, by the late 1800s, settlers had cleared as much as 80–90% of the land in the northern Chesapeake Bay watershed for agriculture and timber (Cooper, 1995). This interval is marked by an abrupt decrease in *Quercus*, or oak pollen, and a sharp spike in *Ambrosia*, or ragweed, and can be associated with a four- to seven-fold increase in sedimentation rates (Cooper and Brush, 1991; Cooper et al., 1993; Brush, 2001).

Modern bottom water temperatures in the bay vary seasonally, generally ranging from approximately 2 °C–25 °C (Cronin et al., 2003). Based on ostracode Mg/Ca paleotemperatures studies, average temperatures during the late and early Holocene were 14.2 °C–12.8 °C, respectively (Cronin et al., 2003).

2.2. Mulinia lateralis

Mulinia lateralis, a small shallow-burrowing coot clam, is one of the most abundant and well-preserved mollusks recorded in sediment cores from the Chesapeake Bay (Canuel et al. in press). Several thousand specimens can be preserved in a 4 cm thick shell layer. *M. lateralis* shells are aragonitic in composition (Carter, 1990) and range in size from millimeters to centimeters. This species is able to tolerate a wide range of temperature (7.5 °C–32.5 °C), salinity (0–80 ppt), and oxygen conditions and inhabits latitudes from 10 to 55°N (Calabrese, 1969; Winn and Knott, 1992; Daley, 2002). *Mulinia* is considered an opportunistic species because it can colonize quickly after an ecologic disturbance and survive when other species are unable (Daley, 2002), and thus it may be indicative of poor environmental conditions (Dauer and Alden, 1995; Weisberg et al., 1997).

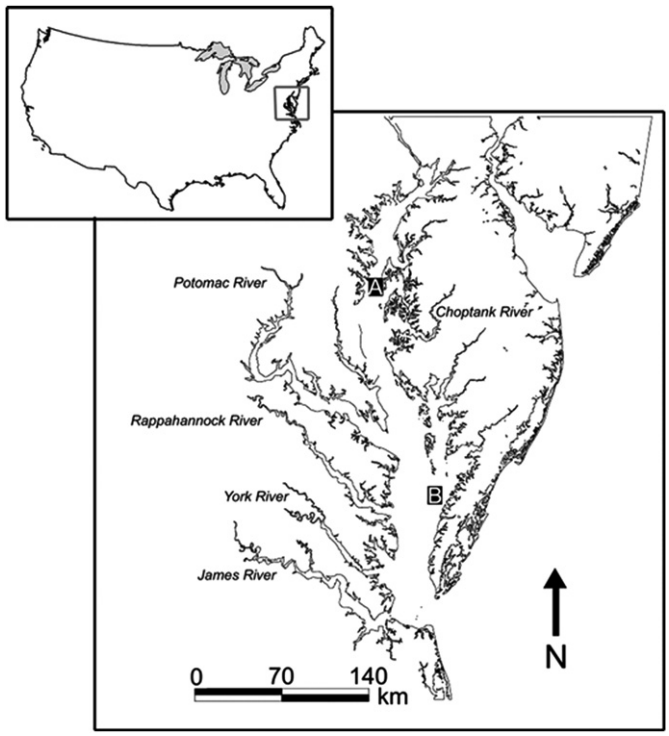

Fig. 1. Map of core locations in the Chesapeake Bay, USA. Two cores were sampled: (A) MD03-2661 drilled near Kent Island (38°53.21′N; 76°23.89′W) in 25.5 m of water and reaching a core depth of 24.48 m and (B) MD03-2656, collected near the Pocomoke River (37°43.25′N; 75°56.51′W) in 16.3 m of water and reaching a core depth of 16 m.

2.3. Sediment cores and sampling

Samples were collected from two cores, recovered via Calypso piston coring by the U.S. Geological Survey in June 2003: (1) MD03-2661, drilled near Kent Island (38°53.21′N; 76°23.89′W) in 25.5 m of water, reaching a core depth of 24.48 m and (2) MD03-2656, collected near the Pocomoke River (37°43.25′N; 75°56.51′W) in 16.3 m of water, reaching a core depth of 16 m (Fig. 1). Full details of these cores are available at http://geology.er.usgs.gov/eespteam/Atlantic/overview.htm.

Cores were refrigerated at the U.S. Geological Survey in Reston, Virginia, and sectioned into 2 cm core segments. The subsections were rinsed in deionized water through a 63 μm sieve, and the coarse residue was dried overnight at 50 °C. *Mulinia* shells were collected under a 3× magnification microscope, the majority extracted from the >150 μm sediment size portion using a fine brush.

MD03-2661 was the primary focus of this study, as it is one of the longest and most complete cores ever recovered from the Chesapeake Bay. Preliminary dating by the USGS, which included 20 radiocarbon dates from *M. lateralis* shells, revealed a hiatus between 1088 cm and 1217 cm (2800 and 5600 cal yrs BP, see Willard et al., 2005; Cronin et al., 2007). The average sedimentation rates above and below the hiatus for MD03-2661 are 0.31–0.36 cm per year, respectively, based on radiocarbon dating. In the MD03-2661 core, a minor increase in *Ambrosia* occurs at 189 cm, which represents the very first land clearance circa 1600 AD (D. Willard, personal communication 2011).

The MD03-2656 core was chosen to assess the applicability of the ^{14}C-AAR calibration curve created from the MD03-2661 data. Preliminary dating for this second core includes five radiocarbon-dated *Mulinia* with calibrated ages from 8000 to 9000 yrs BP and another three shells with "modern" radiocarbon ages at depths of 68.5, 148–158, and 205 cm (see Willard et al., 2005; Cronin et al., 2007) The sedimentation rate for MD03-2656 between 8000 and 9000 cal yrs BP is approximately 0.2 cm per year. Assuming Holocene bottom water temperature differences between the two core sites have been constant, the equation from the direct calibration of articulated shells should also be applicable to the MD03-2656 core.

3. Materials and methods

3.1. Amino acid racemization

328 *M. lateralis* shells were prepared for AAR analyses following the methods of Kaufman and Manley (1998). First, shells were weighed and cleaned with deionized (DI) water using gentle sonification for several minutes. The samples were leached with 2 M HCl to remove 10% by weight, rinsed in DI water and dried under a laminar flow hood. Samples were sealed under nitrogen and hydrolyzed at 110 °C for 6 h. Samples were dried overnight in a vacuum desiccator and then rehydrated with the internal standard, L-homoArginine, which is used to calculate the relative concentrations of the amino acids.

All analyses were performed at Northern Arizona University using a Hewlett–Packard HP1100 reverse phase liquid chromatograph (RPLC) with Chemstation software version A.06.01. The

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HP1100 was equipped with a quaternary pump, vacuum degasser, auto-injector, and HP1046A programmable fluorescence detector (Kaufman and Manley, 1998). An O-phthaldialdehyde/N-isobutyryl-L-cysteine (OPA/IBLC) derivatizing reagent that consisted of 170 mM OPA and 260 mM IBLC was added to the rehydrated samples to make the amino acids fluoresce once in the detector. The fluorescence detector used a xenon-arc flash lamp that responded to this OPA/IBLC reagent, and the Chemstation software was calibrated with this fluorescence. A user-defined macro for Chemstation provided an output with the height, area, and D/L values based on area for several amino acids (see Appendix A from Kaufman and Manley, 1998 for macro). This study focused primarily on aspartic acid (Asx) because it was well-resolved, showed the least variability of the amino acids examined, and racemized relatively quickly for this time scale and at this temperature.

3.2. Radiocarbon dating

Radiocarbon analyses of *Mulinia* samples were conducted by two laboratories: (1) the National Ocean Sciences AMS (NOSAMS) sample preparation lab at Woods Hole Oceanographic Institution (NSF Cooperative Agreement number, OCE-9807266) and (2) Beta Analytic, Inc. (Miami, Florida). All ^{14}C results and calibrated ^{14}C ages used in this study are provided in Appendix A.

The radiocarbon dates for the indirect calibrations of MD03-2661 and MD03-2656 were compiled from previously published work by Willard et al. (2005) and Cronin et al. (2007) and include samples analyzed by both NOSAMS and Beta Analytic. See Willard et al. (2005) and Cronin et al. (2007) for a detailed explanation of sampling and radiocarbon analyses.

All of the *Mulinia* samples selected for direct calibration were visually examined as for AAR analyses and subjected to gentle sonication in deionized water. Standard laboratory procedures for radiocarbon analysis were employed (http://www.whoi.edu/nosams/home). Other than visual (microscope) examination, data sampling, and data screening discussed below and in Edwards (2007), no criteria for selection of the radiocarbon samples were applied.

Calibrated radiocarbon ages for all of the dates used in this study were calculated using the IntCal09 calibration curve (CALIB 6.0; www.calib.org; Reimer et al., 2009). The Marine09 dataset (http://calib.qub.ac.uk/marine/) does not include any data for the Chesapeake Bay itself, only for nearby shelf samples. Colman et al. (2002) dated three museum specimens of the oyster, *Crassostrea virginica* from the Chesapeake Bay, to derive a regional marine reservoir correction (ΔR) of 365 ± 143 yrs, which is not statistically different from the typical 400 year marine reservoir, so a ΔR of 0 was used for this calibration.

3.3. Calibration of AAR with ^{14}C

Calibration of AAR with radiocarbon was accomplished using three different approaches: (1) direct calibration with articulated shells, (2) direct calibration with split shells, and (3) indirect calibration. To our knowledge, this is the first study to employ and compare three independent metrics for calibration. The approach is further strengthened by the use of direct calibration with samples, in which valves (or portions of valves) from the same specimen are used for calibration (e.g., Wehmiller et al., 1995; Sloss et al., 2004; Barbour Wood et al., 2006; Kosnik et al., 2008; Krause et al., 2010). Direct calibration contrasts with indirect calibration, in which samples from the same core intervals (but not the same specimens) are compared (e.g., Kaufman et al., 2008).

The first approach used in this study, direct calibration with articulated shells, involved sampling eight fully articulated specimens of *M. lateralis*, disarticulating them, and selecting one valve for AAR analysis and the other valve for radiocarbon dating. For the second approach, direct calibration using split shells, seven individual valves of *M. lateralis* were split in half bilaterally, one portion was analyzed for AAR, while the remaining portion was sent for radiocarbon analysis. Shells that exhibited relatively high or low Asx D/L values for their respective depth intervals were preferentially selected for this calibration. While this sampling approach allowed us to evaluate variability in Asx D/L values, it inevitably produced a noisier calibration curve (see Results below). For the third approach, indirect calibration, independent radiocarbon dates were provided by the USGS for particular intervals within the core, then 129 individual *Mulinia* valves from the same intervals were submitted for AAR analysis. Once a calibration model for core MD03-2661 was established using each of the three methods, these models were compared to each other, and the direct calibration model with articulated shells was applied to *Mulinia* from a second core MD03-2656. It should be noted that the resulting age models are only applicable to Chesapeake Bay *Mulinia* from the Holocene, and that models will likely vary for other temperature and environmental regimes (Mitterer, 1993; Wehmiller, 1993; Wehmiller and Miller, 2000).

3.4. Mathematical racemization models

Over the years, authors have used various types of functions to calibrate AAR data with radiocarbon. For example, a simple linear fit may be used if considering slower racemizing amino acids and taxa or if investigating a relatively short time scale (Goodfriend, 1997; Clarke and Murray-Wallace, 2006). Because statistical tests generally assume normally distributed linear data, studies have transformed both time (usually by using the square root of age, see Goodfriend et al., 1992; Sloss et al., 2004; Kidwell et al., 2005) or D/L (by raising it to some power, such as Goodfriend et al., 1996; Barbour Wood et al., 2006; Kaufman, 2006; Kosnik et al., 2007, 2008). For a more in depth review, see Clarke and Murray-Wallace (2006) and Kaufman (2006).

In the past decade, authors have begun using the simple power function, $y = m(D/L)^x + b$ (see references cited above). In a recent paper by Kosnik et al. (2008), the authors fit their data through $y = m(D/L)^x + b$, where y is cal radiocarbon age (years before 1950), D/L is the ratio of the D- to L-amino acid, b is the y-intercept or live D/L, and m and x are the parameters determined by minimizing the regression residuals. Kosnik et al. (2008) created seven distinct calibration curves with radiocarbon for aspartic and glutamic acids with each taxon (*Ethalia*, *Natica*, *Tellina*, and *Turbo*) resulting in 56 calibration curves, and concluded that weighting had no influence on the calibration curve, but that including the live-collected D/L was critical.

3.5. Sources of AAR variability

The three independent calibration approaches make it possible to evaluate several of the factors that increase AAR variability. Variability of AAR ratios has been recognized for decades with studies of Pleistocene samples (Miller and Hare, 1980; Wehmiller and Belknap, 1982; Miller and Brigham-Grette, 1989; Wehmiller, 1993; Wehmiller and Miller, 2000), but these sources must also be considered for their Holocene counterparts, namely: (1) analytical error, (2) secondary contamination, (3) intrashell variation, (4) inherent (intershell) variability, (5) intergeneric variability, and (6) time averaging or age mixing. This study is either able to eliminate or estimate each of variability sources, depending on which calibration is applied, as explained below.

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Analytical error is defined as variability in amino acid values resulting from sample pre-treatment, preparation, and chromatography. In other words, submitting the exact same sample through the AAR process multiple times would produce a slight range of D/L values, the variability of which would represent analytical error. For this study, the analytical error of Asx D/L, estimated with replicate RPLC analyses of interlaboratory comparative standards as a measure of CV is ≤1.9% (Kaufman and Manley, 1998; Kosnik et al., 2008).

The influence of secondary contamination in the study system is inferred by high levels of serine, an unstable amino acid that declines rapidly in abundance during early diagenesis (Miller and Brigham-Grette, 1989). Contamination with modern amino acids means these samples will tend to have lower D/L values than their cohorts (Kaufman et al., 2008). In this study, secondary contamination is controlled for (i.e., assumed to be 0%) via data screening prior to calibration, by studying (1) the ratio of L-Ser to L-Asx (here referred to as "Ser/Asx"; Kaufman, 2006; Kosnik et al., 2008) and (2) variability and covariance of Asx and Glx D/L ratios.

Intrashell variability is defined as within-shell variability, and is produced when different portions of the same valve are analyzed for AAR (e.g., Miller and Hare, 1980; Miller and Brigham-Grette, 1989). One can choose to sample from the same part of the shell (e.g., hinge, anterior, posterior) or analyze whole shells when possible (Goodfriend et al., 1997). Intrashell variability can be remarkably high, ranging from 10 to 30% for Holocene brachiopod and mollusk shells, and is much more substantial for some species and ages than others (Goodfriend et al., 1997; Carroll et al., 2003). For the purposes of this study, intrashell variability is assumed to equal 0% when whole valves are analyzed. If this assumption is incorrect, and intrashell variability for the articulated and indirect calibrations is greater than 0, it follows that our estimates of intershell variability and time averaging will be reduced.

Following the terminology of Hearty et al. (2004), we explicitly consider inherent variability to be intershell variability caused by post-mortem diagenetic alteration of organic matrix that occurs even if shells of the exact same age were deposited together at the same time and in the same environment. In heating experiments, which are designed to simulate natural racemization, two living shells of the same species that are heated under identical conditions will yield a slight scatter of D/L values, usually within 2–3% (Goodfriend, 1991; Goodfriend and Meyer, 1991; Kaufman, 2006). For earlier studies correlating Pleistocene sediments, multiple D/L values are averaged to create an aminozone, and the inherent variability of any aminozone would include the effects of age mixing (see, for example, Miller and Hare, 1980; Wehmiller and Miller, 2000; Wehmiller et al., 2010).

Intergeneric variability is the well-documented observation that different genera have distinctly different rates of racemization (Lajoie et al., 1980; Wehmiller, 1980; Sloss et al., 2004; Kidwell et al., 2005; Kosnik and Kaufman, 2008; Kosnik et al., 2009). This phenomenon is easily controlled for by confining a study or calibration to a single genus and species, in this case *M. lateralis*.

All of the aforementioned variability influences must be considered before undertaking an estimate of age mixing or time averaging. Simply put, time averaging occurs when fossils of non-contemporaneous organisms are preserved together (Olszewski, 1999). Rates of biological accumulation tend to be higher than sedimentation rates, creating a tendency toward time averaged assemblages (Martin, 1999). In addition, processes such as physical reworking, bioturbation, and chemical dissolution compound this effect, and result in mixing on the order of hundreds to thousands of years that has been observed in the majority of sedimentary deposits studied (Kidwell and Flessa, 1995; Wehmiller et al., 1995; Martin et al., 1996; Kowalewski et al., 1998; Olszewski, 1999).

Several of these factors are minimized across our study, since our analysis is limited to a single species in a deep channel estuarine environment that varied approximately 2 °C in temperature throughout the Holocene (Cronin et al., 2003). Moreover, our use of three calibration approaches allows us to further control for different sources of variation for each calibration. For example, the direct calibration with articulated shells is not affected by intrashell variability (since whole valves are used) or time averaging (since valves of articulated specimens do not vary in age), but they are affected by inherent variability. The direct calibration with split shells involves analyzing different portions of the same valves, and therefore is not affected by time averaging (since different portions of the same valve do not vary in age), but is affected by intrashell (since bilateral splitting of shells may be inexact) and inherent variability. Finally, the indirect calibration is not affected by intrashell variability (since whole valves are used), but is affected by inherent variability and time averaging. If we assume that these sources of variability do not differ substantially among calibration datasets, it should be possible to apply the estimated analytical error to the direct calibration with articulated shells to calculate the overall inherent variability for Holocene *Mulinia*. Then this estimate of inherent variability could be used to estimate intrashell variability (using the direct calibration with split shells) and time averaging (using the indirect calibration) for this study system. All statistics presented herein were performed using OriginPro 7.5 (OriginLab, Northampton, MA) or SPSS 19.

4. Results

4.1. AAR screening criteria

A myriad of screening criteria have been employed in the literature (for recent review see Kosnik and Kaufman, 2008). For this study, two metrics were applied to screen the data prior to calibration: (1) levels of serine concentration and (2) variability of the covariance of Asx and Glx D/L values.

4.1.1. Serine concentration

Serine is an amino acid that breaks down rapidly, thus its presence in older samples can indicate secondary contamination (Miller and Hare, 1980; Miller and Brigham-Grette, 1989; Murray-Wallace et al., 2001). Recent studies using the same analytical methods employed here have suggested a cut-off of 0.8 for L-Ser to L-Asx ratios (here referred to as "Ser/Asx") (Kaufman, 2006; Kaufman et al., 2008).

For the *Mulinia* analyzed in this study, 96% of samples recorded Ser/Asx ratios of less than 0.3 (Fig. 2). In general, Ser/Asx ratios decrease and become more variable as sample weight decreases (Fig. 2). For the MD03-2661 data set, a more conservative cutoff of 0.7 for the Ser/Asx ratio was applied, and two samples were excluded (OS-53047 in Table 1 and 1615–40 in Appendix B). Coincidentally, both samples with high Ser/Asx values also had anomalous Asx–Glx covariance, thus two criteria independently call for exclusion of these results (see Section 4.1.2 and Fig. 3).

4.1.2. Variability of covariance of Asx and Glx D/L ratios

There are two ways to consider the covariance. A common method for assessing the reliability of a given D/L ratio is to examine internal consistency, which is simply the covariance of two amino acids such as Glx and Asx (Hearty et al., 2004; Kaufman et al., 2008). Descriptive statistics were performed for each core interval using SPSS v.19, then each individual was analyzed for the difference from its respective mean. Recent studies have argued that any samples that plot beyond a 2σ (Kaufman, 2003; Hearty

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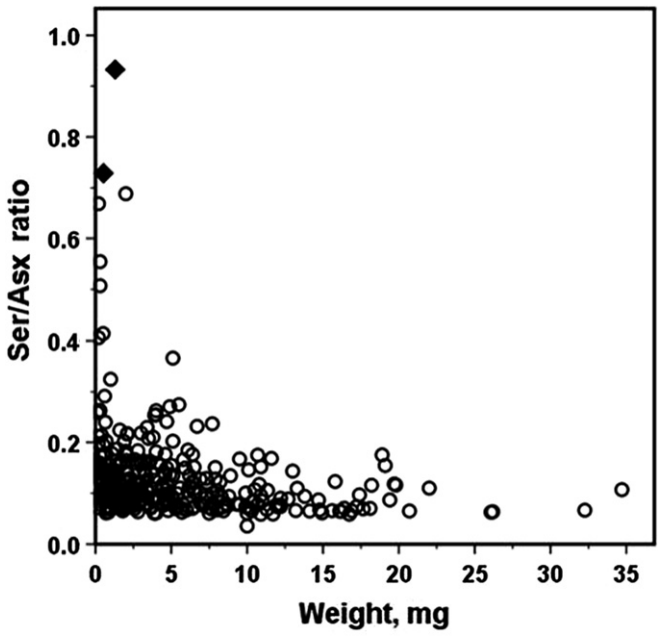

Fig. 2. Relationship between the ratio of L-Ser to L-Asx (referred to as "Ser/Asx") and sample weight for all specimens analyzed from core MD03-2661. High values of Ser/Asx ratios are associated with small shells, but only two specimens (black diamonds) fell above the 0.7 cut-off and were removed prior to calibration.

et al., 2004) or 3σ (Kosnik and Kaufman, 2008) envelope of Asx and Glx D/L covariance should be considered outliers.

There are two distinct ways in which each sample can vary from the mean. First, if only Asx or Glx D/L is 2σ beyond the interval mean, a sample lacks the internal consistency such that neither of its D/L values is considered reliable (Hearty et al., 2004; Kaufman, 2006; Kosnik and Kaufman, 2008). The second possibility is that both Asx and Glx D/L values are 2σ beyond the interval mean, implying that this sample may be significantly older (or younger) than its cohorts. Such samples, called "outliers D/L values" in Kosnik and Kaufman (2008), should be removed before calibration.

For this data set, all 329 *Mulinia* shells sampled from the MD03-2661 core showed covariance between Asx and Glx D/L (non-linear regression to fit an exponential curve, $r^2 = 0.883$, $p < 0.0001$, $n = 329$; Fig. 3). Only two samples fell beyond 2σ for both Asx and Glx, and another 18 samples were considered aberrant due to their lack of Asx–Glx covariance (see Table 1 and Appendix B). When the 22 outliers identified above were eliminated due to Ser/Asx ratio or Asx–Glx relationships, the r^2 value for Asx–Glx covariance increases to 0.896 (non-linear regression to fit an exponential curve, $p < 0.0001$, $n = 307$).

4.2. Live shells

Ten live-collected shells were sampled in 2006 from the mouth of the York River (Fig. 1) and then submitted for AAR analysis (Appendix C). The average Asx D/L value of these samples was 0.034, and the standard deviation was 0.001, yielding a CV of 3%. Kosnik et al. (2008) reviewed the common ways to incorporate live D/L values into a calibration, suggesting that if live data are available, they can be used either as a fixed *y*-intercept or as another numerically dated sample with their age as the year of collection. We chose the latter. The average Asx D/L value of the live shells is included in all subsequent calibrations with an age of −56 cal yrs BP, because radiocarbon ages are expressed as cal yr BP, meaning before 1950 which is considered "pre-bomb" (Reimer et al., 2009).

Furthermore, because all ten of the samples were collected live and are therefore known to be the same age, these data also control for age mixing. The resulting 3% CV therefore represents the combined effects of analytical error (<2%) and inherent variability within the live-collected samples. Because these results were from living shells, we assume no diagenetic alteration has modified the shells and their constituent amino acids.

4.3. Direct calibration with articulated shells

Eight articulated *Mulinia* were collected from four intervals from the MD03-2661 core: 371, 598, 1061, and 1615 cm (Table 1, Fig. 4A). Although numerous regression models have been suggested in the literature for AAR calibration, a power function (Hearty et al., 2004) was applied here because: (1) it has proven useful in past studies focusing on aspartic acid and (2) it provided the best fit for all three calibrations. Although Manley et al. (2000) advocated the use of an exponential curve for Asx in mollusks, in this particular case, a power curve provided the best fit. Applying a power curve to the articulated shell data produced the following function:

Table 1
Raw data for direct calibration with articulated shells and split shells for Kent Island core (Core MD03-2661; latitude 38°53.21′N; longitude 76°23.89′W; 25.5 m water depth).

	Lab #	Depth of ^{14}C date (cm)[a]	Weight (mg)	Asx D/L	Glx D/L	Ser/Asx ratio	δ^{13}C	^{14}C age conv	1σ	Cal ^{14}C age (yrs BP[b])	2σ ^{14}C (yrs BP[b])
Articulated	OS-50449	371	5.6	0.153	0.036	0.1	−1.0	1280	35	826	907–733
	OS-50538	371	4.6	0.169	0.043	0.1	−0.8	1340	40	883	968–774
	OS-50539	598	7.3	0.200	0.043	0.1	−0.6	1830	30	1366	1465–1292
	OS-50540	598	4.8	0.192	0.041	0.1	−0.5	1850	40	1391	1499–1299
	OS-50669	1061	3.6	0.237	0.053	0.1	−0.3	3010	95	2793	3045–2527
	OS-50676	1061	0.7	0.251	0.051	0.1	−0.6	2760	90	2495	2716–2295
	OS-50541	1615	4.0	0.317	0.068	0.1	−0.3	5870	45	6289	6389–6194
	OS-50542	1615	11.7	0.321	0.071	0.1	0.3	6130	45	6562	6672–6435
Split	OS-52106	1150	5.7	0.227	0.059	0.1	−0.3	3610	40	3506	3618–3395
	OS-53034	1150	2.0	0.314	0.115	0.1	0.1	4000	80	4012	4256–3795
	OS-52107	1155	11.9	0.237	0.073	0.1	0.1	3460	30	3343	3426–3253
	OS-52097	1155	2.8	0.314	0.065	0.1	0.1	5080	45	5440	5555–5315
	OS-52300	2028	1.9	0.398	0.136	0.2	−2.8	7440	50	7901	8002–7788
	OS-53033	2028	1.4	0.359	0.134	0.2	−1.2	6740	120	7262	7483–6985
	OS-52248	2075	2.8	0.380	0.139	0.2	−2.8	7140	45	7611	7700–7515
	OS-53047[c]	2075	1.3	0.267	0.069	0.9	−2.3	7310	130	7777	8023–7530

[a] The average depth of each core interval is provided for simplicity.
[b] OS-# refers to samples analyzed at the National Ocean Sciences AMS sample preparation lab at Woods Hole Oceanographic Institution. Calibrated radiocarbon ages were calculated using the IntCal09 calibration curve (CALIB 6.0; www.calib.org; Reimer et al., 2009).
[c] Excluded due to high Ser/Asx ratio.

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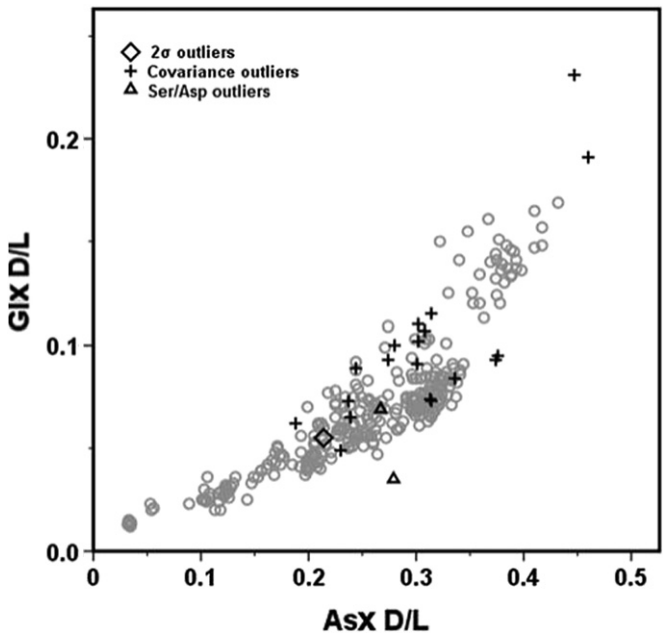

Fig. 3. Plot of Asx D/L versus Glx D/L data for all specimens analyzed from core MD03-2661. Note the positive covariance between the two amino acids. Covariance outliers were samples that lacked internal consistency such that either Asx D/L or Glx D/L is 2σ beyond the interval mean are considered unreliable. 2σ outliers were those where both Asx D/L and Glx D/L were beyond the interval mean. It should be noted that these outliers fall outside the 2σ envelope for their particular core interval, rather than across all core intervals. Hence, they will not necessarily plot as an outlier in this particular plot. Samples with Ser/Asx ratio >0.7 are indicative of modern contamination. All of these types of outliers were excluded from calibration for a total of 22 samples (7% of total).

$$\text{Asx D/L} = 0.034 + 0.00936(56 + t)^{0.39} \quad (1)$$

in which t is the calibrated age (years before 1950), 0.034 is the average Asx D/L of the live data, and −56 is the collection year of the live samples (2006 AD). Radiocarbon date and Asx D/L have a strong positive covariance and are statistically significantly related to each other (non-linear regression to fit a power curve, $r^2 = 0.98$; $p < 0.05$, $n = 8$). Because the direct calibration of articulated shells is not affected by intrashell variability (since whole valves are used) or time averaging (since valves of articulated specimens do not vary in age), this method should provide the most accurate age model of the three approaches used and is the one we recommend using for future applications of AAR to chronology of Chesapeake Bay sediments.

4.4. Direct calibration with split shells

Thirty-three *Mulinia* valves were sampled from four intervals of the MD03-2661 core (1150, 1155, 2028 and 2075 cm), bilaterally split in half, after which one portion was analyzed for AAR. The remaining portions of the valves with the highest and lowest Asx D/L values from each interval ($n = 7$) were then sent for radiocarbon analysis (Table 1, Fig. 4B). This approach provided a useful test of Asx D/L ratio variability, since the relative age predicted by AAR was confirmed with radiocarbon in each instance (Fig. 4B). An eighth shell, from interval 2075 cm, has a Ser/Asx ratio of 0.9 and so was not considered in the calibration (see Table 1). The following power curve function was obtained for the data from split shells:

$$\text{Asx D/L} = 0.034 + 0.00154(56 + t)^{0.606} \quad (2)$$

in which t is the calibrated age (years before 1950), 0.034 is the average Asx D/L of the live data, and −56 is the collection year of the live samples (2006 AD). Although Asx D/L and radiocarbon are significantly, positively correlated to each other (non-linear regression to fit a power curve, $r^2 = 0.893$; $p < 0.05$, $n = 7$), the r^2 value is not nearly as high as that obtained from the direct calibration of articulated shells. This is not a surprise, given the sampling strategy outlined above. The drawback to sampling the highest and lowest Asx D/L values is that it artificially inflates the variation around the regression line. In addition, although the valves were split as carefully as possible, intrashell variability introduces some variation into the results along with analytical error and monospecific intershell variability. These problems, compounded by the lack of sampling of specimens younger than 2800 BP, make this the weakest of the three calibrations reported.

4.5. Indirect calibration

Independent radiocarbon dates were provided by the USGS for nine intervals in the MD03-2661 core (including 371, 598, 713, 1053, 1088, 1217, 1413, 1615, and 2028 cm). Between 7 and 45 individual *Mulinia* valves ($n = 129$) were then sampled from each of the same nine intervals for AAR analysis (Appendix B, Fig. 4C), then averaged to yield a single value for each interval ($n = 9$). The indirect calibration curve yields similar results to Eq. (1) derived from the direct calibration with articulated shells (Fig. 4A):

$$\text{Asx D/L} = 0.034 + 0.0127(56 + t)^{0.356} \quad (3)$$

in which t is the calibrated age (years before 1950), 0.034 is the average Asx D/L of the live data, and −56 is the collection year of the live samples (2006 AD). Once again, the radiocarbon and Asx D/L data have a strong positive covariance and are significantly related (non-linear regression to fit a power curve, $r^2 = 0.99$; $p < 0.05$, $n = 9$), which is also stronger than the direct calibration with split shells. Although the indirect calibration is not affected by intrashell variability (since whole valves are used), it is affected by inherent variability and time averaging.

4.6. Comparisons of calibration curves

The 95% confidence intervals overlap for the direct calibration using articulated shells and the indirect calibration, suggesting that the models are statistically indistinguishable (Fig. 5A). The direct calibration using split shells, in contrast, falls outside the confidence intervals for the direct calibration using articulated shells (Fig. 5B) for the youngest and oldest segments of the core. This is most likely due to the narrow range of core intervals sampled (1150–2075 cm) for the direct calibration using split shells and the fact that we have no samples in that dataset younger than ~2800 years.

4.7. Quantifying sources of AAR variation

Once the three calibrations were established, they were then used to quantify the different sources of AAR variability affecting these data (Table 2). We assumed that these sources (e.g., analytical error, intrashell variability, intershell variability, and time averaging) would not be correlated with mean values and would therefore be best expressed as coefficients of variation (CV) of the D/L ratios. We also assumed that the sources of variability within data sets were independent of each other and, therefore, could be summed within each calibration data set (i.e., articulated, split, and indirect) to allow us to estimate unknown variances given our knowledge of known variances.

The direct calibration with articulated shells is affected by two main sources of variability: analytical error and monospecific

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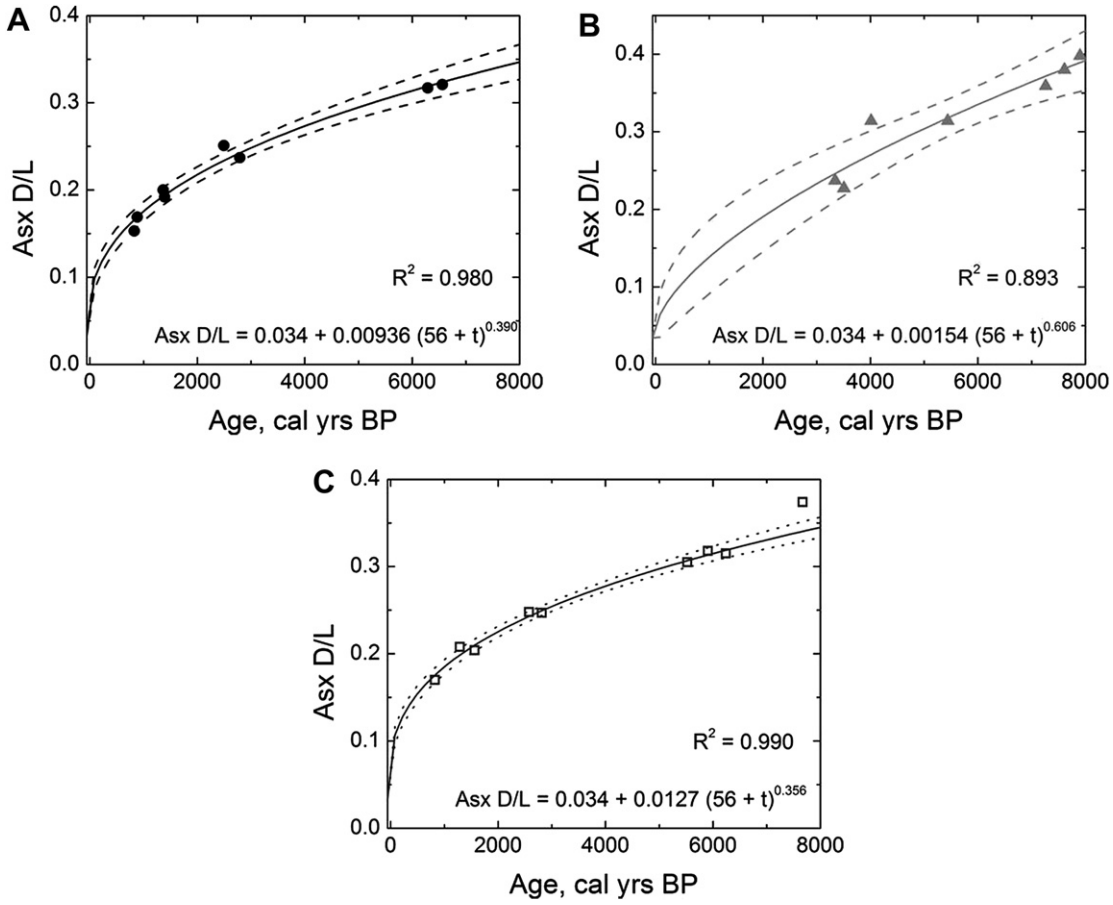

Fig. 4. Screened data and calibration curves for core MD03-2661 produced using three different approaches: (A) Direct calibration with articulated shells. A power curve with 95% confidence intervals was used to describe the calibration between ^{14}C calibrated age and Asx D/L values for eight articulated specimens, (B) Direct calibration with split shells. A power curve with 95% confidence intervals was used to describe the calibration between ^{14}C calibrated age and Asx D/L values for seven split specimens sampled. (C) Indirect calibration. A power curve was used to describe the calibration between ^{14}C calibrated age and Asx D/L values for 129 specimens averaged within each of the 9 core intervals.

intershell variability (=inherent variability). To recreate the variance attributable to analytical error (estimated to be 2% CV), we assumed that analytical error induced, on average, 1 SD of difference from the predicted model. By applying a 2% CV of analytical error, data points were recreated (i.e., 2% deviation of predicted Asx D/L values given the best fit power curve) around the modeled power curve. We used the difference between these and the calibration curve to compute an unbiased estimate of the total variance (i.e., sum of squared differences, divided by sample size minus one) attributable to analytical error. By applying this methodology to the direct calibration using articulated shells, we estimated the unknown variance due to intershell variability to be equal to the

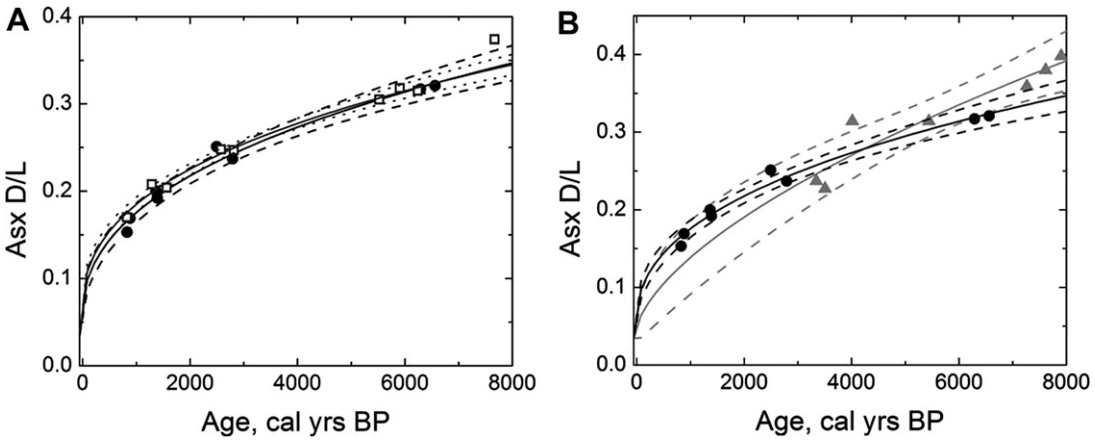

Fig. 5. Comparison of three calibration curves. A. Direct calibration of articulated shells with 95% confidence intervals (black circles and black dashed lines) versus indirect calibration with 95% confidence intervals (open squares and gray dotted lines). Overlap of the 95% confidence intervals demonstrate that the curves are not statistically significantly different from each other. B. Direct calibration of articulated shells with 95% confidence intervals (black circles and black dashed lines) versus direct calibration of split shells with 95% confidence intervals (gray triangles and gray dashed lines). Note that the curves diverge significantly from each other in the youngest and oldest portions of the core.

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Table 2
Assumed or estimated sources of variability for the three calibration approaches in this study.

	Articulated	Split	Indirect
Analytical error	2%	2%	2%
Intrashell variability	0%	7%	0%
Intershell variability (inherent)	3%	→	→
Time averaging (age mixing)	0%	0%	7%

total variance in the data minus the variance due to analytic error. We computed the square root of the variance due to intershell variability to estimate the standard deviation and divided by the average Asx D/L value in the data set (multiplied by 100) to estimate the overall CV. This process led to an estimate of CV for intershell variability of approximately 3%, indicating that intershell variability is similar in magnitude to analytic error (Table 2). It is important to emphasize that, if intrashell variability is greater than 0 in the articulated and indirect calibrations, then intershell variability will in fact be less than 3%. Our estimate therefore represents a maximum estimate for intershell variability.

This estimate of monospecific intershell variability, when combined with our direct calibration using split valves, allows us to estimate intrashell variability. It must be noted, however, that using this particular calibration to assess variability is somewhat problematic, given the dearth of samples from the top of the core and the sampling strategy used. It is therefore likely that these estimates of intrashell variability are exaggerated. We estimated the variance associated with intrashell variability, by subtracting the total variance present in the direct calibration using articulated shells (representing the sum of variances due to analytical error and intershell variability) from the variance present in the direct calibration using split shells. We then calculated the square root of the estimated intrashell variability and divided by the mean of Asx D/L values in the same data set to estimate CV explained by intrashell variability. These computations resulted in an estimate of CV of intrashell variability of 7%, indicating that intrashell variability may extend as high as twice that of intershell variability.

Our original estimate of monospecific intershell variability can also be used to estimate time averaging (e.g., age mixing) using the data from the indirect calibration. Following the same general protocol as before, we subtracted the total variance in the direct calibration using articulated shells from the total variance in the direct calibration using split shells to estimate the variance due to time averaging. From this, we took the square root and divided by the mean Asx D/L value in the direct calibration using split shells to estimate CV due to time averaging. This technique rendered an estimate of CV due to time averaging of 7%, suggesting that the variability in the data due to time averaging is similar to that due to intrashell variability. Our estimate is likely to be an upper estimate, since we have assumed that intrashell variability is 0 in the articulated and indirect calibrations.

4.8. Comparison to core MD03-2656

Although it is a shorter core with fewer occurrences of *Mulinia*, MD03-2656 provides an important test of the applicability of the MD03-2661 calibration curves. These cores are assumed to have experienced similar temperature histories throughout the Holocene, and thus the calibration curve derived for MD03-2661 should be applicable to MD03-2656. If confirmed, it will demonstrate the use of aminochronology as another valuable geochronologic tool for the bay.

Data from core MD03-2656 were screened in the same manner as MD03-2661: checking for Asx–Glx covariance, 2σ Asx–Glx outliers, and Ser/Asx ratios. No samples plotted outside of the 2σ envelope for their interval (possibly due to the low sample size of 4–6 shells per interval). All MD03-2656 samples recorded Ser/Asx ratios of 0.2 or less.

Although the data are too limited to derive an independent indirect calibration curve for MD03-2656, the direct calibration with articulated shells and the indirect calibration raw data and curves from MD03-2661 overlap substantially with the MD03-2656 data. The oldest radiocarbon dates for MD03-2656 are comparable to the MD03-2661 data, but the amount of missing time is more extensive for the former (Appendix D, Fig. 6). All MD03-2656 specimens sampled to a core depth of 205 cm were classified as "modern" (less than 400 years old) by radiocarbon dating. No *Mulinia* shells were recovered between 205 and 644 cm and shells between 644 and 851 cm were dated to between 8000 and 9000 cal yrs BP via radiocarbon (Appendix D). The increased standard deviation for the oldest interval of MD03-2656: 728–730 cm is mirrored in that of the oldest radiocarbon dated samples of the MD03-2661 core (Fig. 6).

5. Discussion

5.1. Use of data screening criteria

After screening the data for high Ser/Asx ratios and Asx–Glx covariance, only 7% of data were removed prior to calibration.

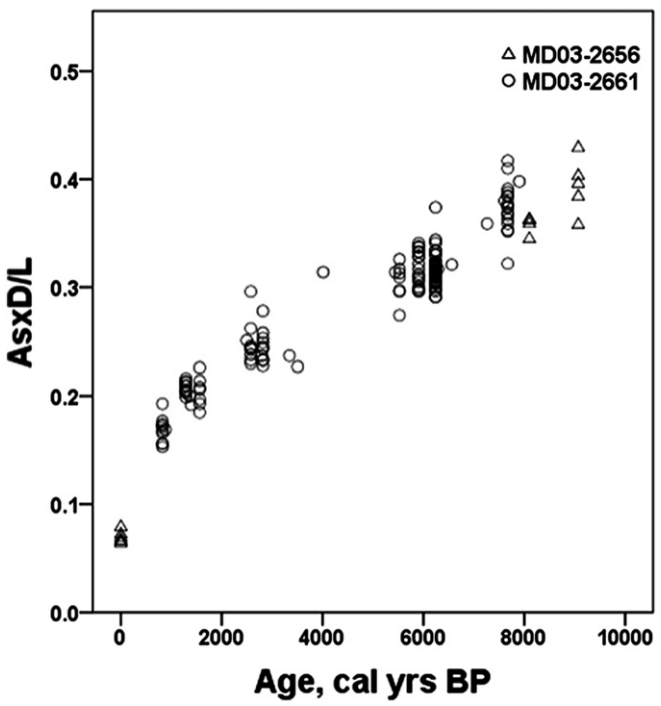

Fig. 6. Screened data and indirect calibration curve for MD03-2661 (open circles) plotted with screened data from MD03-2656 (open triangles). Although samples from MD03-2656 are limited and molluscan material only occurs toward the top and bottom of the core, the 2656 samples do overlap well with the MD03-2661 calibration and display similar levels of Asx D/L variation with the MD03-2661 shells. This suggests that a single calibration model based on Holocene *Mulinia* may be applicable to different sites in the bay.

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This attests to the high reliability of *Mulinia* data from the Holocene of the Chesapeake Bay. Removal of these data does not substantially affect the indirect calibration model, and two of these data points should be reintroduced for subsequent time averaging studies, since they are considered internally consistent because both samples were 2σ outliers for both Asx and Glx amino acids, and are thus regarded as potentially reworked or time averaged specimens (Kaufman and Miller, 1992). Those points that were excluded for high Ser/Asx ratios or because either Asx or Glx were beyond 2-sigma would not be reincorporated, because these amino data are fundamentally unreliable (Hearty et al., 2004).

Ser/Asx ratios of >0.8 are commonly used as a cut-off, but in this case 0.7 appeared to be a more appropriate cut-off for this dataset. Even so, only two samples were removed from analysis based on Ser/Asx ratio. In fact, only six samples from MD03-2661 produced Ser/Asx ratios above 0.5, again demonstrating the high reliability of these *Mulinia* data. Moreover, although many studies have incorporated much smaller fossils (ostracods, foraminifera), the smallest *Mulinia* in this dataset tend to have more variable and higher Ser/Asx ratios, possible due to their high surface area to volume ratios (see Appendix in Hearty et al., 2004).

5.2. Advantages of multiple calibration techniques

One of the most promising results of this study is the close correspondence between the direct calibration with articulated shells and the indirect calibration. Even though the influences of inherent variability and time averaging are superimposed on the indirect data, the two equations are not substantially different. This may be, in part, because the indirect calibration was calculated using mean Asx D/L ratios for each of the nine core depths, thereby reducing the variability within the dataset. The similarity between the two calibrations suggests that when articulated shells are difficult or impossible to obtain, an indirect calibration with an adequate number of shells (in this case 30–40 averaged across 9 core depths) spanning the entire time range of interest should suffice. If these options are unavailable, the split shell and MD03-2656 datasets demonstrate how to interpret incomplete records. Neither the direct calibration with split shells nor the MD03-2656 data were able to produce adequate calibrations independently, however, because these datasets were not complete for the younger portions of the cores.

The MD03-2656 data illustrate that even a core with limited molluscan material, sampled from a completely different region of the bay, produces data that fit the calibration for the much more complete, high sedimentation MD03-2661 core. The MD03-2661 calibration also works well for MD03-2656 data, in spite of the slight ($\sim$0.5 °C) bottom water temperature difference between the sites. Although additional testing of the calibrations will be beneficial, the combined calibrations presented here should provide reliable a geochronological tool for the Holocene sedimentary record of Chesapeake Bay, particularly for samples less than 400 years old that cannot be assessed using radiocarbon.

5.3. Causes of AAR variability: time averaging

Although sources of AAR variability are often mentioned, few geochronological studies attempt to parse variability into its different sources. Somewhere in between the relatively minor effect of analytical error and the oft-studied problem of time averaging, lie the complications of intrashell and inherent variability. Although not easy to define or tease apart, elimination of the latter from estimates of time averaging is vital.

If we estimate time averaging using 1 SD (Kowalewski et al., 1998), this 7% CV translates into an estimate of time averaging of 197 years (range = 394) for the youngest shells in the dataset (which are approximately 826 years old) and an estimate of 1308 years (range = 2616) for the oldest shells in the data set (which are approximately 6562 years old). It is important to emphasize that this is a generalized estimate throughout the core and does not reflect the fact that age mixing undoubtedly varies from one core interval to another. On the one hand, our elimination of Asx/Glx outliers is likely to artificially decrease our estimates of time averaging. On the other hand, if the assumption that sources of AAR variability are independent of each other is violated, then our estimates of time averaging are likely to be inflated.

Applying the same approach to our radiocarbon dataset is problematic, since it would require us to calculate standard deviation based on only two samples ($n = 2$ per core interval). Not surprisingly, the radiocarbon yields even lower estimates of time averaging. Estimates (1 SD) range from approximately 36 years (range = 57) for the youngest shells in the data set (which are approximately 826 years old) to approximately 130 years (range = 260) for the older shells in the data set (which are approximately 6562 years old). The minimum estimate was 13 years (range = 25) for core depth 598 cm (approximately 1379 years ago), while the maximum was 1049 years (range = 2097) for core depth 1155 cm (approximately 4392 years ago). The complexities of age mixing, changes in age mixing with core depth, and radiocarbon estimates of age mixing will be addressed in detail in a later paper.

6. Conclusions

The age models produced in this study represent the first calibrated aminochronology for Holocene sediments of Chesapeake Bay. Now that the calibration has been established, amino acid racemization can provide an alternative to costly radiocarbon dating for samples in this estuary. Although the calibrations are influenced by several sources of variability, the direct calibration of articulated shells and the indirect calibration yielded virtually identical age models. Specifically, we recommend the use of the direct calibration using articulated shells, because this calibration avoids variability due to intrashell variability and time averaging, and yields an extremely high r^2 value. This age model is now applicable to other *Mulinia*-bearing cores in the bay, as demonstrated by the results for core MD03-2656. The calibration design for this study allowed us to estimate several sources of AAR variability, including analytical error (2%), intrashell variability (7%), inherent variability (3%), and time averaging (7%).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.quageo.2012.06.005.

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