AMINO ACID RACEMIZATION DATING OF SOME COASTAL PLAIN SITES, SOUTHEASTERN VIRGINIA AND NORTHEASTERN NORTH CAROLINA

By

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ABSTRACT

Abstract

Bivalve mollusks (g. *Mercenaria* and g. *Anadara*) have been analyzed from 4 southeastern coastal plain sites using the amino acid racemization (AAR) technique for correlation and age estimate purposes. As seen previously (Wehmiller and Belknap, 1982), a conflict exists between age estimates obtained by AAR and U-series methods at the Norris Bridge, VA locality. Here, U-series dating of coral yields a 187 $\pm$20 KA age, while AAR methods suggest a 400 to 500 KA age. The following methods are employed to understand this discrepancy at the Norris Bridge site: 1). Analysis of standard samples to ensure uniform sample preparation and system operation; 2). Quantification of variation in *Mercenaria* and *Anadara* samples for each field site; 3). Qualitative comparison of AAR behavior between *Mercenaria* and *Anadara* to assess suitability of *Anadara* for dating purposes; 4). Aminostratigraphic correlation of field sites using both genera, and 5). Estimation of AAR ages using the the non-linear model of Wehmiller and Belknap (1982).

Standard samples analyzed for this study show relatively good precision. Coefficients of variation (CVs) of D/L leucine values are 5.7%

(ILC-A) and 9.1% (ILC-B). Overall, both ILC-A and -B show accuracy within 6% when compared to other University of Delaware analyses. Several valves of each genus were analyzed from all sites to determine field site precision. CVs for multiple *Mercenaria* samples from each site are as follows: Gomez Pit, VA (5.2%); New Light Pit, VA (7.0%); Moyock Pit, N.C. (8.8%) and Norris Bridge, VA (4.4%). CVs for *Anadara* samples are slightly higher, but comparable for each field site. No *Anadara* were analyzed from the New Light Pit locality. On the basis of an identical order of racemization rates (i.e. ASP$\geq$ALA$>$LEU$>$VAL) and similar ASP/LEU values in both genera, *Mercenaria* and *Anadara* qualitatively show similar racemization behavior.

3 clusters of D/L leucine values have been discerned from these field site analyses. These D/L leucine values are used to correlate fossiliferous strata in the southeastern Virginia and northeastern North Carolina Using the non-linear kinetic model, these Coastal Plain. D/L values suggest the following ages: 0.21 to 0.25 (75 KA) and 0.530 (approximately 400 KA). D/L values ranging between 0.33 and 0.38 are tentatively estimated to be 100 to 200 KA in age.

CHAPTER 1

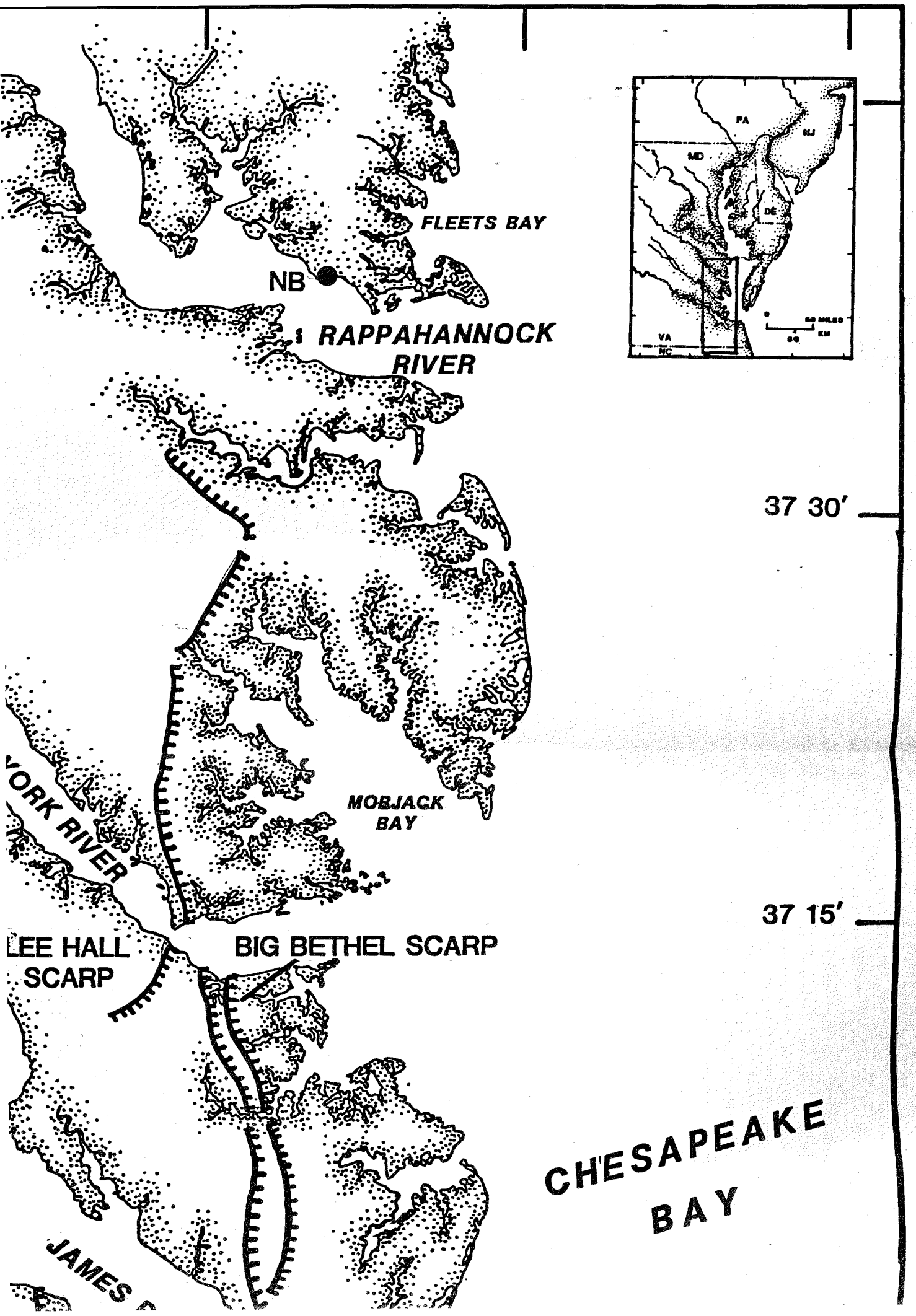
INTRODUCTION

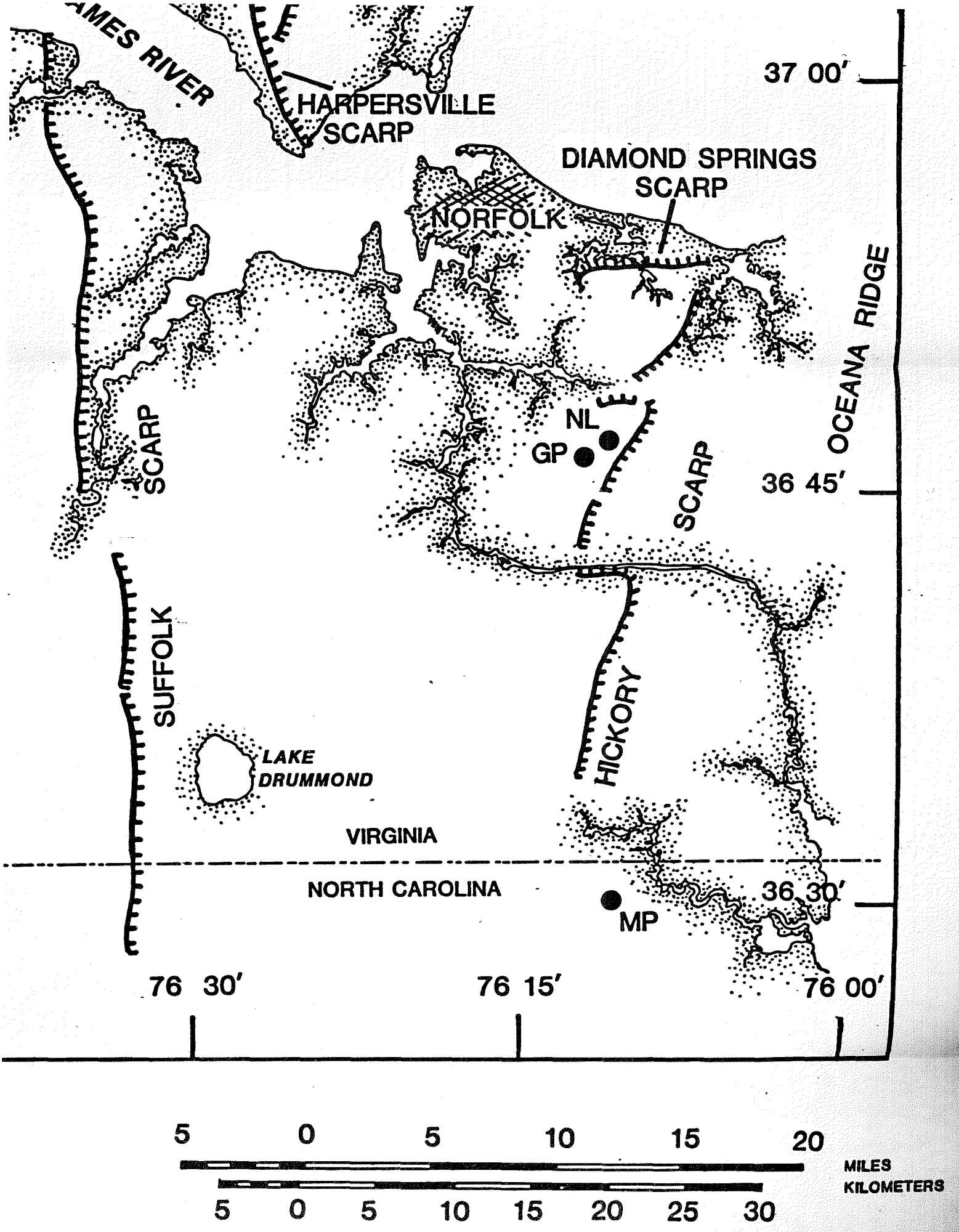
1.1 Statement of Purpose

A conflict exists between age estimates obtained by the amino acid racemization dating method and U-series dating of corals at the Norris Bridge, Virginia locality (Wehmiller and Belknap, 1982). D/L leucine values from several *Mercenaria* analyses suggest an age greater than that indicated by the 187 ±20 KA U-series coral date (Cronin *et al.*, 1981). If D/L leucine values support the 184 ±20 KA U-series coral date at Norris Bridge, a significantly warmer climate over time is inferred. This conclusion is not consistent with palynologic and microfaunal evidence for the southeastern Coastal Plain region (Valentine, 1971; Cronin *et al.*, 1981). Additionally, calibrating amino acid enantiomeric ratios with the 187 ±20 KA U-series coral date at the Norris Bridge site would result in serious conflict with existing non-racemic D/L leucine values from older units in warmer climates (Wehmiller and Belknap, 1982).

This study is an attempt to understand the discrepancy between U-series and amino acid age estimates at the Norris Bridge site, and to estimate ages for other field sites in the region using both *Mercenaria* and *Anadara*

Figure 1-1: Area of Study. NB=Norris Bridge, NL=New Light Pit, GP=Gomez Pit, MP=Moyock Pit.


FLEETS BAY
NB
RAPPAHANNOCK RIVER
37 30′
MOBJACK BAY
YORK RIVER
37 15′
LEE HALL SCARP
BIG BETHEL SCARP
CHESAPEAKE BAY
JAMES
PA
NJ
MD
DE
VA
NC
KM


AMES RIVER
HARPERSVILLE SCARP
37 00′
DIAMOND SPRINGS SCARP
NORFOLK
OCEANA RIDGE
NL
GP
SCARP
36 45′
SCARP
SUFFOLK
HICKORY
LAKE DRUMMOND
VIRGINIA
NORTH CAROLINA
36 30′
MP
76 30′
76 15′
76 00′
5 0 5 10 15 20
MILES
5 0 5 10 15 20 25 30
KILOMETERS

D/L data. The following methods are employed:

1. Quantification of precision and accuracy of standard samples to ensure uniform sample preparation and system operation.

2. Statistical analysis of *Mercenaria* and *Anadara* field samples on several levels. Chromatographic, sample and locality precision are quantified.

3. Qualitative comparison of relative racemization rates between *Mercenaria* and *Anadara*, in order to assess the suitability of *Anadara* for dating purposes.

4. Aminostratigraphic correlation of field sites using *Mercenaria* and *Anadara* D/L data.

5. Estimation of amino acid racemization ages using the non-linear kinetic model (Wehmiller and Belknap, 1982).

Possible reasons for the conflict between U-series and amino acid age estimates at the Norris Bridge site include an analytically questionable U-series date or incorrect temperature assumptions (Wehmiller and Belknap, 1982). Each of these problems is considered in this study.

1.2 The Non-linear Kinetic Model

A non-linear model (Fig. 1-2) for racemization reflects the diagenetic pathway of proteins in molluscan shells (Wehmiller, 1982 and references therein). Over time, shell proteins hydrolyze, liberating the constituent amino acids. Racemization of these amino acids takes place during the entire process of hydrolysis, but at different rates dependent upon the position of the amino acid in the peptide chain. It has been shown that terminal amino acids racemize at a faster rate than interior residues due to steric hindrance and R-group effects in the latter (Smith and Evans, 1980 and references therein). As a result of changing racemization rates during amino acid diagenesis, a non-linear kinetic curve is used to most accurately assign age to a D/L value. The non-linear curve is divided into 3 components (Fig. 1-2): two linear segments of differing slope, and a transition zone. The first linear component shows a rapid racemization rate, probably due to the presence of extensively racemized terminal amino acids produced during rapid hydrolysis of shell proteins. The second linear segment shows a lesser slope, representing a slower racemization rate. Here, free amino acids predominate and are already racemized, or will invert at a slower rate. The transition zone is poorly characterized kinetically. This zone is believed to be related to the breakdown of high molecular weight polypeptides in fossil shells (Wehmiller, 1982).

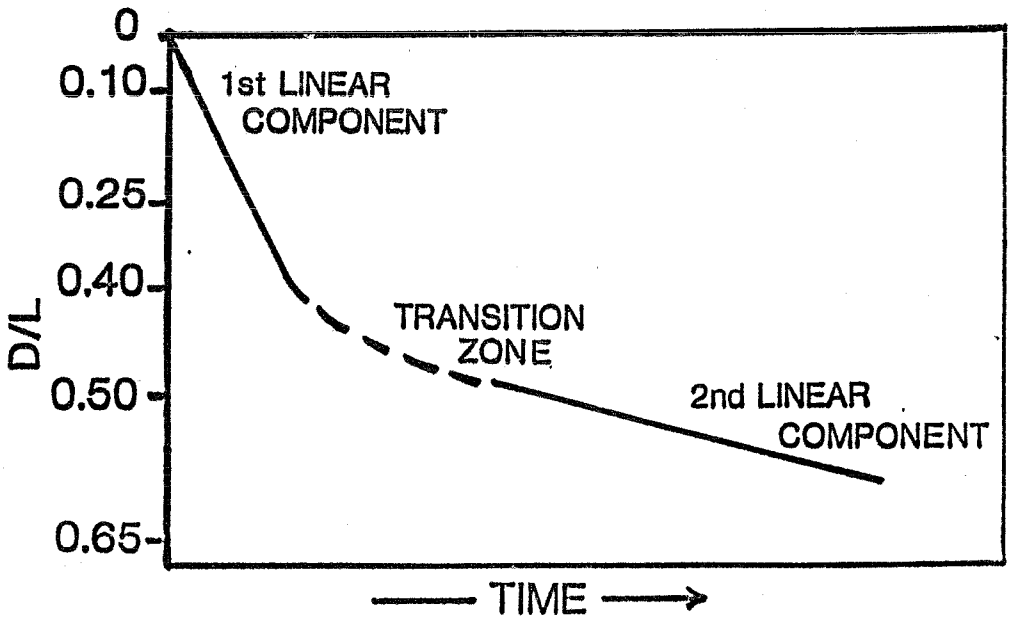


Figure 1-2: The Non-Linear Kinetic Curve for the Racemization Reaction. Redrawn from Wehmiller (1982).

The racemization reaction shows a logarthmic relationship between temperature and reaction rate. Rate constants have been determined for the epimerization of isoleucine (Bada and Schroeder, 1972; Mitterer, 1975) and these rate constants are used to estimate the temperature dependence of the leucine racemization reaction.

Both timing and magnitude of temperature change must be considered when interpreting D/L leucine values in a geochronologic context. These qualities of temperature change may best be approximated using the Effective Quaternary Temperature (EQT) model, supplemented by micropaleontological evidence. The EQT is used to model temperature history for a region in light of the glacial-to-interglacial climate fluctuations which characterize the Pleistocene Epoch. The EQT is the temperature calculated from the weighted average of rate constants for samples of a given age. Warmer climatic periods exert a disproportionally greater effect upon racemization rate than do cooler climatic periods of equal duration. Fossils older than 128 KA (Stage 5e) have endured more than one complete glacial-to-interglacial cycle. As a result, rate constants for warm and cool periods "average out" and EQT values for samples older than 125 KA are all within approximately 1° of each other (Wehmiller and Belknap, 1982). Further discussion of this model can be found in Chapter 7 (this thesis), Wehmiller (1982), and Wehmiller and Belknap (1982). EQT calculations made in this study (App. G) were made following those shown in Appendix C of Belknap (1979).

CHAPTER 2

METHODS

2.1 Sample Collection and Preparation

Certain criteria are recognized for the collection of unaltered shell samples in the field. Shells should be *in situ*, preferably articulated. Samples showing chemical and mechanical weathering in the form of bleaching, leaching and encrustation with metal oxides must be avoided as they are more likely to yield irreproducible results. Ideally, the shell should not have been exposed. The presence of a well-preserved proteinaceous hinge ligament on an articulated specimen is an indication of good preservation. After collection, shells to be analyzed are rinsed in tap water, then traced onto paper and labelled for identification purposes. Specimens were only identified to the genus level in this investigation.

In the lab, individual valves are sampled. 1g fragments are cut from the hinge area with a Foredom dental saw equipped with a carborundum wheel. Periostracum, and inner lamellar and outer prismatic layers of the shell are removed as best as can be determined through visual inspection. For the most reproducible results, only hinge samples are used for analysis (Chapter 5.2.2).

Selected mollusk samples from each site are analyzed by x-ray diffraction to determine % calcite recrystallization. These spectra are used as guides to judge the condition of each sample location as a whole. Generally, any sample which did not appear to be excellently preserved was suspect and not used. % Calcite recrystallization was analyzed using the method of Turekian and Armstrong (1960) and found not to exceed 2% (samples 83NB-126, 83NB-26, 83GP-69 and 83MP-131 were analyzed).

2.2 Wet Chemical Methods

Laboratory techniques described here reflect development and refinement over a number of years through work performed at the Amino Acid Laboratory at the University of Delaware. The following is a succinct description of the standard method employed for the analysis of larger shell samples. For a more detailed consideration of variations in this method, reference is made to Belknap (1979) and York (1984).

Because shell fragments are easily subject to contamination, certain precautions must be employed during sample work-up. No sample is handled throughout the procedure without PVC gloves to avoid the addition of fingerprint-borne proteins. Reagents can also be a source of sample contamination. To reduce this possibility, concentrated "ultraclean" HCl and NH_4OH reagents are prepared by bubbling the respective gases through triple-distilled water to saturation. All subsequent dilutions of these reagents

employ triple-distilled water. Reagent blanks are run periodically to ensure purity. Any step in the wet chemical preparation of a sample, including rinse of the packed cation exchange column is performed using triple-distilled water. Organic reagents dichloromethane (DCM), methanol (MeOH) and isopropanol (Iprop) are distilled prior to use. All glassware in contact with the sample proceeding the chemical cleaning step is acid-washed and stored at 300°C for at least 48hr. All other glassware are stored at 150°C following acid-wash.

Chemical cleaning of the shell fragment is accomplished through successive washes of "ultraclean" 1N HCl, triple-distilled water, "ultraclean" 2N NH_4OH, triple-distilled water and finally "ultraclean" 1N HCl and water. These cleaned samples are vacuum-dried and weighed to the nearest 0.001g. The sample is then transferred to an "ultraclean" test tube for dissolution.

The sample is dissolved slowly in the quantity of concentrated "ultraclean" HCl needed to bring the final solution to 6N. Care is taken to avoid vigorous bubbling during the addition of acid to prevent sample loss. Upon complete dissolution, samples are sealed under N_2 in the test tube using an oxygen-propane mixture torch. Samples are now ready for hydrolysis.

The hydrolysis step breaks the peptide bonds of proteins, yielding free amino acid residues. Hydrolysis of sealed samples proceeds for 22hr at

105°C in a heating block. Racemization of the sample during this step is believed to be a maximum 4% (Williams and Smith, 1977). Analysis of modern *Mercenaria* has yielded D/L leucine of 0.015, representing a racemization of 3% (Belknap, App.F; 1979). Further discussion concerning the duration and intensity of hydrolysis, and its effect upon the sample can be found in Belknap (1979).

After hydrolysis, calcium cations are removed from the sample. This desalting step employs Dowex (50W-X8) cation exchange resin. Prior to use, it is prepared in the following manner:

1. Used resin is dumped into a 600ml sintered glass funnel and washed with the following: 4l distilled tap water, 1 funnel volume of 4N HCl, 4l tap distilled water and finally 1 funnel volume of 2N NaOH. Resin is now ready for use in a new cation exchange column.

2. Once poured into a column, the resin is exposed to only "ultraclean" reagents. Experience has shown that a column filled with approximately 10 ml resin is sufficient to desalt a 1g shell sample.

3. Poured columns are subject to further cleaning through the successive addition of 30ml 2N NaOH, 75ml triple-distilled water, 30ml 4N HCl and 75ml triple-distilled water. Columns are now in

the H+ form and ready to desalt hydrolyzed samples.

The hydrolyzed sample is transferred to the column using pipettes stored at 300°C. After the sample drains through the resin, the column is washed with triple-distilled water until the eluate is of neutral pH. Excess H+ and Cl- ions are removed by this wash. 35ml 2N NH_4OH is added to elute anionic amino acids from the column. This mixture is collected in a 100 ml round-bottom flask, and used resin is recycled. Samples are then taken to dryness using a Roto-Vap rotary evaporator. The rotary evaporator is equipped with a glass trap to prevent contamination of the sample by the condensate. Samples are gently heated (40°C) by immersion in a warm-water bath to hasten the drying process. Upon drying, the sample is transferred to an "ultraclean" 14/20 ground-glass derivatization tube through the addition of 1.5ml 1N HCl by pipette. The new mixture in the derivatization tube is again taken to dryness without heat.

Derivatization of $-COO^-$ and $-NH_3^+$ moities is necessary for analysis of sample by gas chromatography (GC). During derivatization, it is necessary to maintain anhydrous conditions, as not to diminish the yield of the ester derivatives. Addition of all reagents occurs within a glove box, under N_2.

The sample to be derivatized is vacuum-dried at 45-50°C for 1hr. 0.75ml to 1.0ml H+-saturated Iprop is added. "Breathing towers" or ground-glass joint tubing fashioned with Dri-Rite plugs are used to maintain

anhydrous conditions in the derivatization tube during the following heating step. Samples and breathing towers are then immersed in a silicon oil bath (105°C) for one hour, and dried down under a stream of N_2. Esterification is now complete.

For the acylation step, 0.75ml to 1.0ml DCM and 0.15ml trifluoroacetic anhydride (TFA) are added, within the glove box. The sample is sealed and allowed to sit for 2hr at room temperature. Samples are again dried under N_2, followed with addition of approximately 1ml MeOH. MeOH will destroy serine, which interferes with the leucine peak on the chromatogram. Samples are sealed and refrigerated for at least 12hr.

After storage, the samples are dried down on the rotary evaporator without heat. In the glove box, 0.5ml DCM is added and the sample is transferred to a screw-top vial using a pipette. The DCM solvent is evaporated under a stream of N_2. Samples are now ready for injection into the gas chromatograph when re-dissolved in an appropriate volume of DCM, estimated from sample size. When not in use, all samples are stored in desiccant-filled glass jars and frozen.

Gas chromatography is performed using a Perkin-Elmer GC Model 3920, equipped with a 25m Chirasil-Val (Applied Science, Inc. State College, PA) glass capillary column and flame-ionization detector. Program conditions are as follows: 12psi He carrier gas, initial temperature 75°C for 16min;

0.5°/min to 140°C, then isothermal for 32min. All peaks of interest are resolved by 120min. Peaks are identified by comparison of peak retention times and peak intensities with known standards. Fig. 2-1 shows a representative chromatogram. Peak heights are measured from chart recorder spectra and compared to peak area and peak height data from the Hewlett-Packard Model 3309A integrator. 3 chromatograms are usually obtained for each sample derivative.

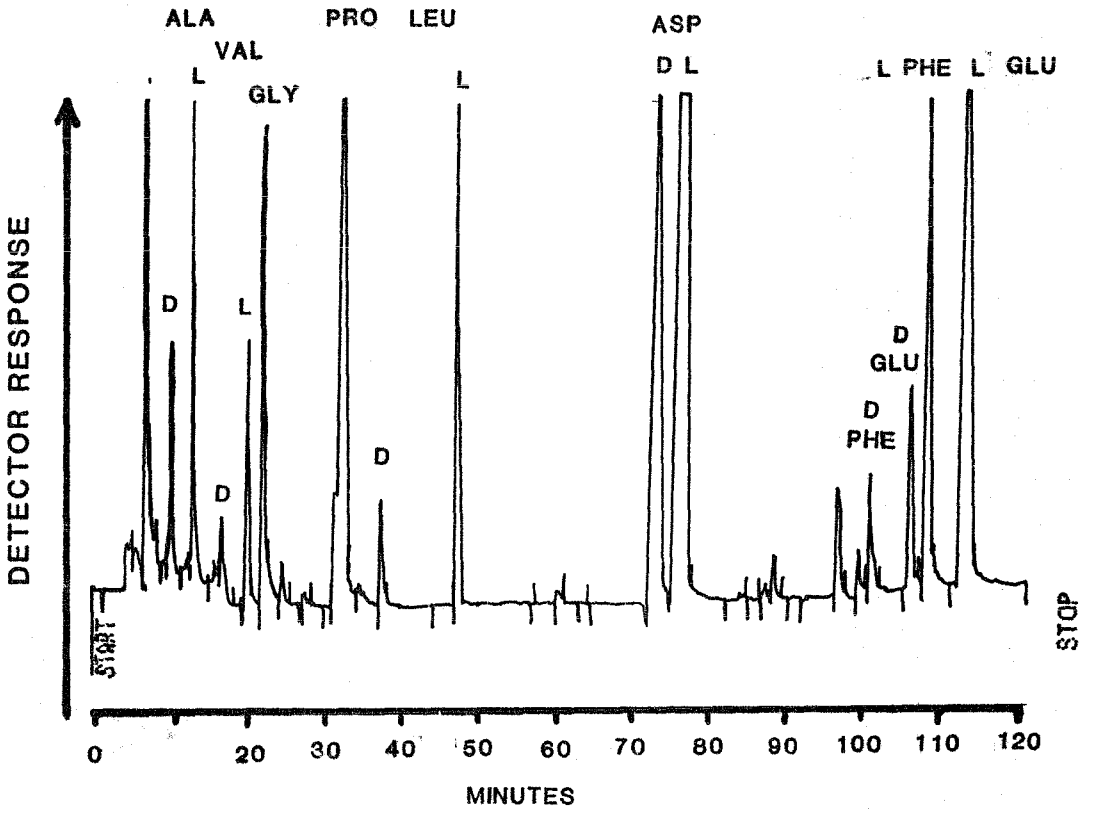


Figure 2-1: Chromatogram for Standard ILC-A Showing D- and L-Amino Acid Peaks

CHAPTER 3

GEOLOGICAL HISTORY

3.1 Regional Setting

Central to the historical development and understanding of the stratigraphy of the southeastern Virginia Coastal Plain is the concept of "terrace-formations" originally proposed by Shattuck (1901). This genetic term is defined as "fluvial, estuarine and marine sediments deposited in a step-like manner" becoming younger and descending in age to the east (Oaks *et al.*, 1974). Each terrace consists of one formation. These terrace-formations are bounded on eastern and western margins by "scarps" which are wave-cut in origin, and considered former shorelines (Flint, 1971; Oaks and DuBar, 1974).

The debate continues as to the validity of the terrace-formation concept, especially when applied to the southeastern Virginia Coastal Plain. Oaks and Coch (1973 and references therein) produced the first detailed lithostratigraphic study of the southeastern Virginia Coastal Plain. Subsequent revisions by these authors (1973, 1974) reiterate the belief that morphostratigraphic classification, i.e. use of the terrace-formation concept, is invalid in this study area. They find 10 post-Yorktown (Plio-Pleistocene) age

lithostratigraphic units separated by unconformities, representing 6 submergent-emergent cycles (Fig.3-1). Table 3-1 shows Pleistocene formations and notes the approximate location of the corresponding shoreline.

FORMATION	LOCATION OF SHORELINE
Emergence	
SANDBRIDGE FM.	Coast-parallel ridges near Va. Beach
LONDONBRIDGE FM.	East of Oceana Ridge
Major Emergence	
KEMPSVILLE FM.	Hickory Scarp
Minor Emergence (?)	
NORFOLK FM.	Suffolk Scarp
GREATBRIDGE FM.	West of present shoreline
Major Emergence	

Table 3-1: Stratigraphic relationships of southeastern Virginia Coastal Plain, as defined by Oaks, *et al.*, 1974

Both Norfolk and Kempsville Formations of Oaks *et al.* (1974) have yielded U-series coral dates, ranging from 62 to 86 KA for the Kempsville Formation (in Oaks *et al.*, 1974) and ranging from 62 to 79 KA for Norfolk Formation corals (in Mixon *et al.*, 1982). These U-series dates suggest Early Wisconsinan to Sangamonian age for these formations.

Mixon *et al.* (1982) propose a revision to the stratigraphic model of Oaks and Coch. With better exposures found at the New Light Pit, VA site, one transgressive sequence is recognized. The Norfolk Formation and the Kempsville Formation represent one Late Pleistocene high sea stand. In addition, they expand the Kempsville Formation to include several facies of

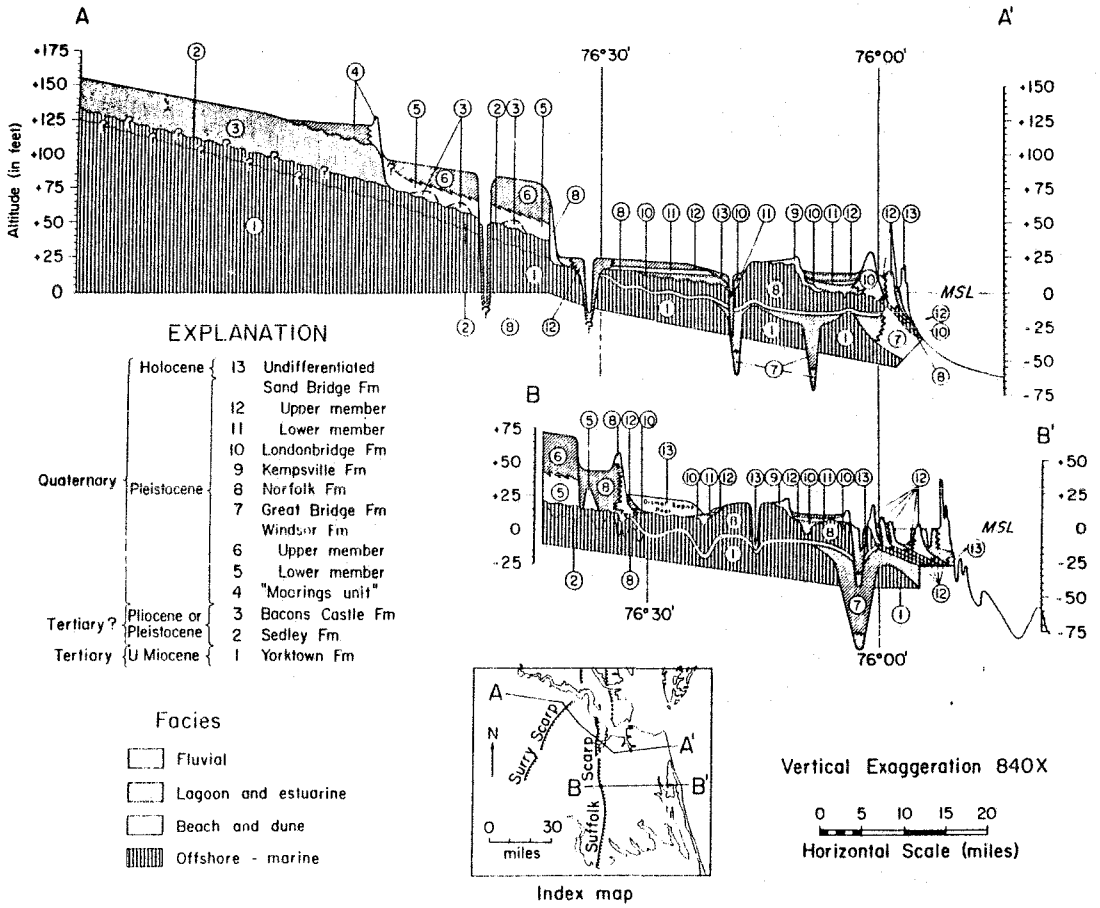


Figure 3-1: SE Virginia Coastal Plain Stratigraphy; After Oaks and Coch, (1973)

the younger Sand Bridge and Londonbridge Formations. Thus, the barrier island sands of the Kempsville Formation are coeval with muddy lagoonal and estuarine facies west of the Hickory Scarp previously ascribed to the Sand Bridge and Londonbridge Formations. True Sand Bridge Formation sediments are restricted to the area north of the Diamond Springs Scarp (Fig. 1-1).

Jasper (1982) and Darby (1983) suggest that the deposits mapped as the Norfolk, Kempsville, Londonbridge and possibly Sand Bridge Formations represent a single regressive-transgressive cycle, rather than 3 cycles as proposed by Oaks and Coch (1973). Furthermore, they suggest that the Kempsville and Sand Bridge Formations be dropped or reduced to member status, and the Norfolk Formation be enlarged and reclassified into 5 facies (Fig. 3-2). This Norfolk through Londonbridge sedimentary package was deposited after a major hiatus, thus rests unconformably upon Great Bridge Formation sediments. Parts of this sediment package are found east and west of the Hickory Scarp. The scarp is a result of deposition during early Norfolk time, and was later incised by tidal streams with the subsequent development of back-barrier lagoon and marsh environments coincident with the Londonbridge sea level rise.

The Poquoson, Lynnhaven and Sedgefield Members of the Tabb Formation (Johnson, 1976) on the York-James River peninsula may be correlative with the Sand Bridge, Kempsville and Norfolk Formations,

respectively, south of Norfolk, VA (Darby, 1983). However, it has recently been suggested that these sediments south of Norfolk can be *reclassified* as the members of the Tabb Formation (Peebles *et al.*, 1984). The significance of the Tabb Formation in the context of a terrace concept will now be discussed.

Most recently, the recognition of terraces as *geomorphic surfaces* has been used to explain the stratigraphy of the southeastern Virginia Coastal Plain (Peebles *et al.*, 1984). In this model, Pleistocene sediments exist in seven "transgressive packages", consisting of fining-upward sequences beneath river and coastal flats (or terraces). These transgressive packages become progressively younger and lower in elevation from east to west. Fig. 3-3 shows a schematic relationship of these transgressive packages. It should be noted that these terraces are not "terrace-formations" as defined previously (p.16). Rather, these terraces represent geomorphic surfaces (or coastal flats) bounded by scarps, possibly underlain by several formations. A complex lithostratigraphy resulted from deposition during several transgressive episodes covering this region.

Generalized facies relationships within each transgressive package are shown in Fig. 3-4 (Pebbles *et al.*, 1984). The Tabb Formation is composed of 3 members: the lowermost Sedgefield Member consisting of bay or lagoonal sediments overlying a basal transgressive pebbly-to-boulder layer. The bay or lagoonal sediments are in turn overlain by cross-bedded barrier

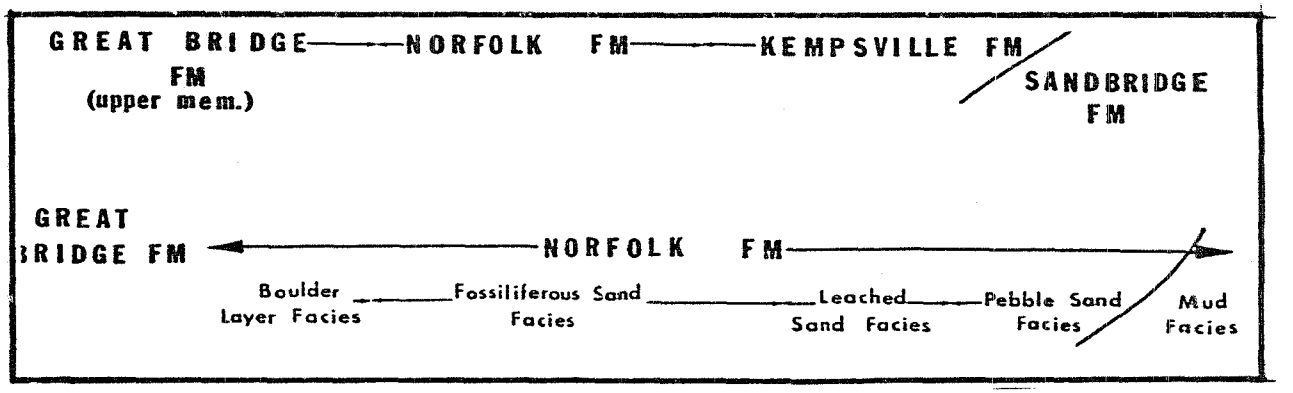


Figure 3-2: Stratigraphy of the Norfolk Formation, from Darby (1983)

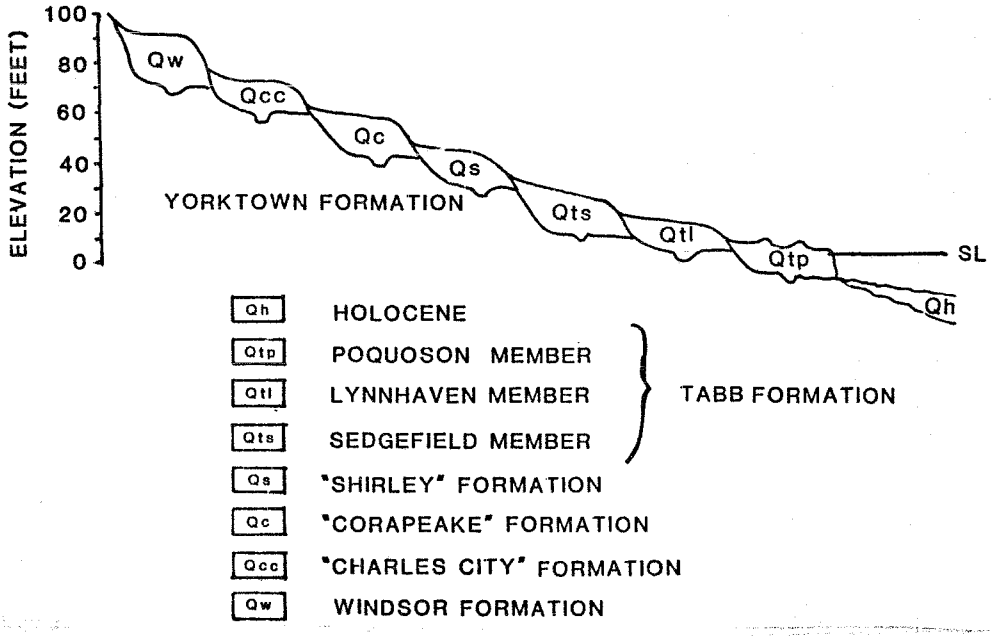


Figure 3-3: The Terrace Model of Johnson *et al.* (1984)

island sands, showing a transgressive sequence as defined by Kraft and John (1979). The middle Lynnhaven Member consists of tidal stream deposits downcutting into Sedgefield barrier sands. This may indicate sea level oscillation or regression. The uppermost Poquoson Member occurs east of the Hickory Scarp and may consist of coast-parallel beach accretion ridges (P. Peebles, pers. comm. 1984).

3.2 Description of Field Sites

Field sites reported in this investigation will be discussed in the context of the proposed stratigraphic models. Aminostratigraphy will be considered in greater detail in Chapter 7.

3.2.1 Gomez Pit

Gomez Pit, owned by the E.A. Williams Paving Co., reveals a stratigraphic section on the Fentress Rise, approximately 1km west of Hickory Scarp (App. F). This section shows estuarine and barrier island sediments, interpreted as a transgressive sequence. Prominent in this section are at least 8 distinct horizons of articulated *Mercenaria* (P. Peebles pers. comm. 1984).

The existence of several superposed, laterally continuous *Mercenaria* horizons permits good aminostratigraphic control between exposures at this site. The excellent preservation and articulated nature of the *Mercenaria* suggest sudden death. Distorted growth lines on *Mercenaria* also indicate

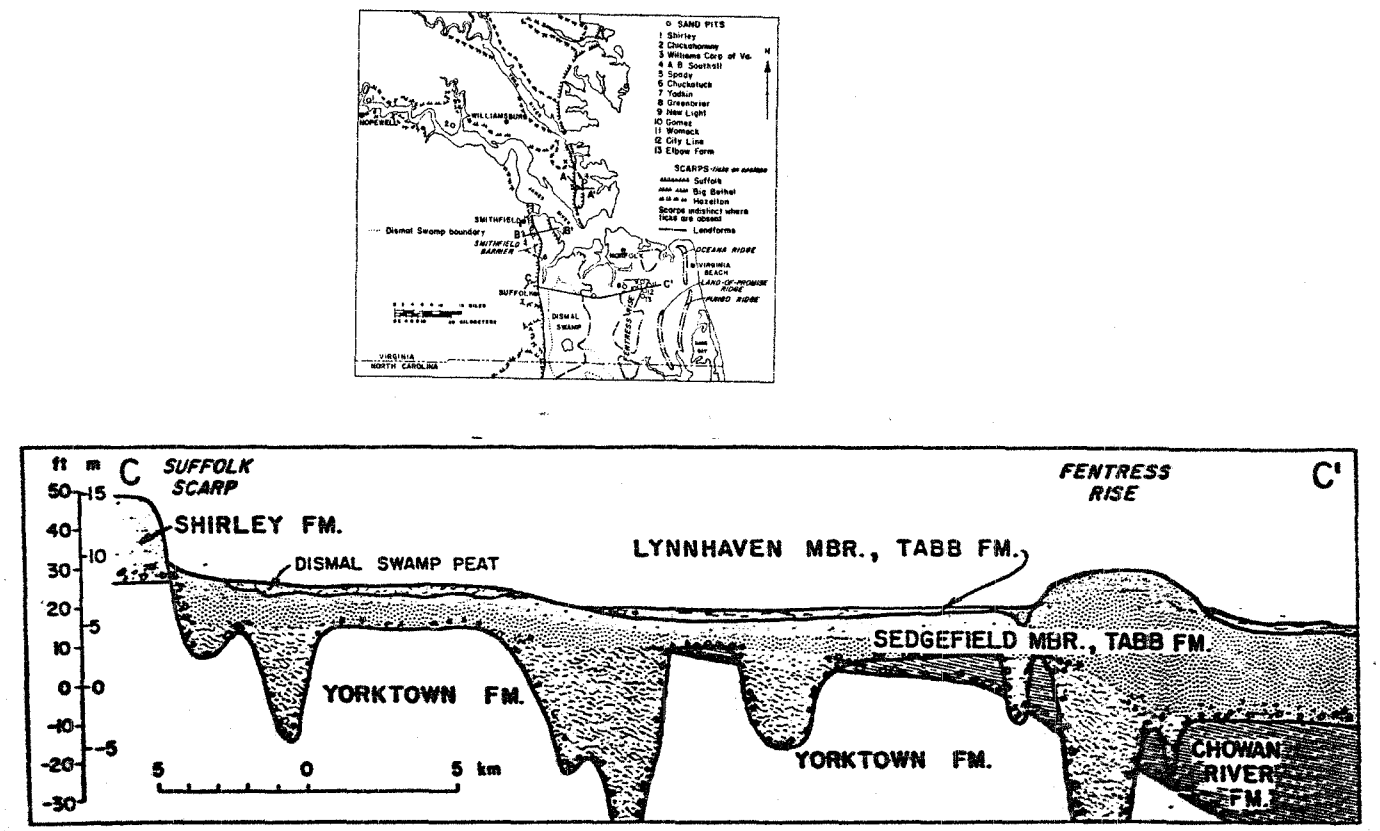


Figure 3-4: Facies Relationships Within the Tabb Formation, from Peebles *et al.*, (1984)

stress, possibly due to freshwater influx (Darby, 1983) or temperature change (Pratt and Campbell, 1974). A complex series of events undoubtably resulted in the formation of these horizons. Ultimately, a barrier island complex migrated over this lagoonal or estuarine environment, marking the maximum transgression for this unit. A regressive phase is shown by stream sediments incised into the barrier island sands, accompanied by the appearance of cypress trees on the stream banks.

A 75 ±5 KA U-series coral date (Cronin *et al.*, 1981) has been obtained from a serpulid zone (Norfolk Formation) within the Gomez Pit (App. F). This date is in general agreement with other U-series coral dates (from Norfolk and Kempsville Formations) in the immediate vicinity. These dates are as follows: 74 ±4 KA (New Light Pit, Norfolk Formation; Cronin *et al.*, 1981); 62-75 KA (4 samples from the Kempsville Formation, Toy Avenue Pit; unpublished data in Appendix G, Belknap, 1979); 59-66KA (4 samples from the Norfolk Formation, in Womack Pit; unpublished data in Appendix G, Belknap, 1979); 62 ±4 KA (Norfolk Formation, Womack Pit; Cronin *et al.*, 1981) and 62-84 KA (4 samples from the Kempsville Formation, Womack Pit; unpublished data in Appendix G, Belknap, 1979). As shown by these U-series coral dates, similar ages have been obtained from specimens within both the Norfolk and Kempsville Formations.

3.2.2 Moyock Pit

Moyock Pit, located just south of the Virginia-North Carolina state line, possibly exposes the same north-south trending sand body seen in Gomez and New Light Pits to the north (Jasper, 1982). Unfortunately, only spoil piles are accessible for collection, and no described stratigraphic section exists. A U-series coral date of 72 $\pm$4 KA (Cronin *et al.*, 1981) has been obtained from this site, and is in agreement with U-series coral dates from both Gomez Pit and New Light Pit.

3.2.3 Norris Bridge

The Norris Bridge site is located on a wave-cut bluff on the north shore of the Rappahannock River (App. F). A transgressive sequence is shown by the up-section transition from estuarine muds to cross-bedded barrier island sands. Within the estuarine sediments are sequential layers of *Rangia*, followed upsection by *Crassostrea* and *Mercenaria/Anadara* layers (App. F). This faunal trend reflects increasingly saline conditions.

Terraces have been geomorphically defined on the upper reaches of the Rappahannock River (Colman, 1983; W.L. Newell, pers.comm. 1983). Belknap (1979) describes this site as existing between two terraces having 12m and 4-7m elevations. However, to apply the geomorphic terrace concept (Peebles *et al.*, 1984) here would be unwise as these "terraces" are poorly

defined topographically, and have only been mapped in reconnaissance (W.L. Newell, pers.comm., 1983). These units are referred to as "Accomack beds" implying age equivalence with the Accomack Formation of the central Delmarva peninsula (Mixon *et al.*, 1982).

U-series dates using both solitary coral (g.*Astrangia*), and bivalve shell (sp.*Mercenaria*) have been reported from the Norris Bridge site. Age estimates obtained are 187 $\pm$20 KA (Cronin *et al.*, 1981) and 139 $\pm$10 KA, (Mixon *et al.*, 1982) respectively. Due to the " open-system" behavior of isotope parent-daughter pairs, a high degree of uncertainty is associated with U-series dating of molluscan shells (Kaufman *et al.*, 1971). Hence, these data will be disregarded. Considering coral age estimates, there are several analytical problems which must be reconciled to verify the 187 KA date.

Criteria for "closed-system" behavior of isotopes have been proposed. These criteria include concordia between $^{230}Th/^{234}U$ and $^{231}Pa/^{235}U$, and a $^{230}Th/^{232}Th$ ratio exceeding 20 (Ku, 1976). In data presented, $^{231}Pa/^{235}U$ ratios were not analyzed for the majority of samples (Mixon *et al.*, 1982) The $^{230}Th/^{232}Th$ activity ratio is 11.0 for the Norris Bridge coral sample (Cronin *et al.*, 1981). Analytically, this age estimate should be considered tentative (Wehmiller and Belknap, 1982).

CHAPTER 4

RESULTS AND DISCUSSION OF STANDARDS DATA

4.1 The Physical Nature of Standard Samples

In order to assess the uniformity of sample preparation by the analyst and consistency of operating conditions of the system, the use and analysis of standard samples is required. Three molluscan standards, ILC-A, -B and -C have been analyzed throughout this study. ILC (Interlaboratory Comparison) samples have recently been distributed worldwide to serve as a reference for future amino acid work (Wehmiller, 1984).

A brief description of standard samples used in this investigation will follow. A complete description of preparation and interpretation of ILC data can be found in Wehmiller (1984). Standards ILC-B and -C are composed of powdered g. *Mercenaria* valves, of 100 to 250 KA and approximately 1 MA in age, respectively. ILC-A is composed of powdered g. *Saxidomus* valves, having an approximate age of 50 KA.

Powdered standard samples are kept frozen, and aliquots are periodically removed as need requires. Samples are analyzed in groups of 6, with 1 standard sample included in each group. This standard is analyzed

routinely on GC, concurrent with the 5 remaining field samples. Statistical data presented in Appendix A is used to determine the precision, or internal consistency of standards personally analyzed, and the accuracy of these data when compared to other University of Delaware standard samples run by other analysts.

4.2 Statistical Considerations: Precision

Statistical analyses of ILC-A and ILC-B standards will be discussed, as these two standards were most often analyzed during this investigation. Amino acid residues showing the best chromatographic resolution and highest precision are alanine and leucine. D/L values for these residues usually show coefficients of variation (CVs) ranging from 1 to 6% for repeat analyses of any one given hydrolyzate (App. A).

When all ILC-A standard D/L data are averaged (21 chromatograms of 5 hydrolyzates), CVs for amino acid residues are as follows: alanine (5.4%), valine (6.1%) and leucine (5.9%). When ILC-B data are considered (22 chromatograms of 4 hydrolyzates) the following CVs are obtained: alanine (2.5%), valine (7.1%) and leucine (9.1%). These data are presented in Appendix A and summarized in Table A-1, pg. 90.

Good precision is shown by ILC-A alanine and leucine, and ILC-B alanine. CVs for these residues are comparable with the 2% to 5% range of

variability reported by other laboratories (Wehmiller, 1984). ILC-B leucine shows poorer precision. The variability between ILC-B D/L leucine values of this study (CV=9.1%) exceeds the published CV value of 5.2% for the ILC-B standard (Table 2; Wehmiller, 1984). D/L valine shows poorer chromatographic precision in both ILC-A and -B standards, as seen by high CVs. Peak interference (Fig. 2-1) may contribute to this lack of reproducibility for valine, as mentioned in Wehmiller (1984).

4.3 Statistical Considerations: Accuracy

D/L values of this study are compared with D/L data compiled from ILC-A and ILC-B standard samples run between January, 1983 through September, 1984 (D. Sirkis, J.F. Wehmiller, L.L. York, unpublished data, 1983, 1984). Also included in this compilation are University of Delaware laboratory D/L data summarized in Table 2 of Wehmiller (1984). These published D/L data summarize standards analyzed between January, 1982 through October, 1982. All data used for this comparison are presented in Appendix A. Tables A-1 and A-2 summarize standards D/L data from this study and from the University of Delaware Laboratory, respectively.

Figures 4-1 (ILC-A) and 4-2 (ILC-B) graphically compare standard mean D/L values for alanine, valine and leucine from each hydrolyzate personally analyzed, with standard mean D/L values compiled from other University of Delaware analyses. Only data from powdered standards

prepared using the isopropanol method are used in this comparison. Table 4-2 shows % Relative Error between these two data sets. Overall comparison of these two data sets shows % Relative Error usually under 5%, except for ILC-A valine. All data used in the compilation of Table 4-2 are found in Appendix A.

SAMPLE	AMINO ACID	D/L VALUE A	D/L VALUE B	%RELATIVE ERROR
ILC-A	Alanine	0.358	0.371	3.63
	Valine	0.123	0.132	7.32
	Leucine	0.191	0.202	5.96
ILC-B	Alanine	0.748	0.727	2.81
	Valine	0.399	0.406	1.75
	Leucine	0.530	0.519	2.07

Table 4-1: Comparison of UD Laboratory Standard Data (A) With JEM-ILC Standard Data (B). All data were obtained using powdered samples and employing the isopropanol method. All standard data are presented in Appendix A. Statistics for (A) and (B) are summarized in Tables A-2 and A-1, respectively.

ILC-B mean D/L leucine values for 3 of 4 hydrolyzates of this study fall beyond the published standard deviation (Wehmiller, 1984) for this residue (Fig. 4-2). This may be due to laboratory overload as low D/L values are reported by other analysts during the period of January and February, 1984 (App. A, pg. 88; J.F. Wehmiller, pers.comm, 1984).

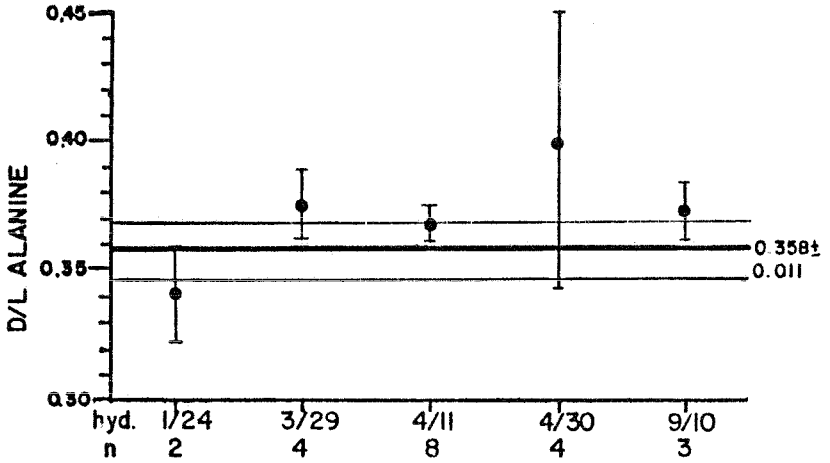
The disparity between these standards data is significant when interpreting field sample data run during the same period. This problem will be further discussed in Chapter 8.

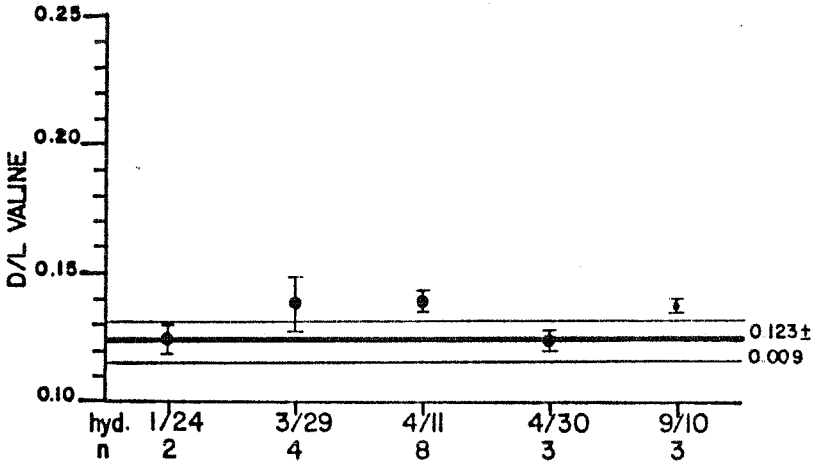
The following conclusions can be drawn from the analysis of standards data:

1. Precision of standard data is determined by averaging all chromatograms for ILC-A or -B and determining the CV for that data set. CVs for ILC-A D/L data are as follows: Alanine (5.4%), valine (6.1%), and leucine (5.7%). ILC-B D/L values show the following CVs: Alanine (2.5%), Valine (7.1%) and leucine (9.1%). ILC-A alanine and leucine, and ILC-B alanine show highest precision.

2. Although standard D/L values personally analyzed show good overall agreement with D/L values compiled from the University of Delaware data of other analysts, there is a discrepancy between ILC-B D/L leucine values between these two data sets. Mean D/L leucine values of 3 hydrolyzates of this study exceed the published CV for this standard, when compared to ILC-B D/L leucine standard data of other University of Delaware analysts. This is significant when interpreting D/L leucine data for field samples analyzed during January and February 1984.

Figure 4-1: ILC-A Standard Data. ILC-A standard hydrolyzates of this study are compared to mean D/L values compiled from Univ. of Delaware standards analyzed between January, 1983 through September, 1984. Also included are Univ. of Delaware data summarized in Table 2 of Wehmiller (1984). Standard deviation of each "grand mean D/L value" from the Univ. of Delaware Laboratory is from Wehmiller (1984). Mean D/L values and standard deviations of hydrolyzates (hyd.) of this study are calculated from "n" chromatograms.


D/L ALANINE
0.45
0.40
0.35
0.30
0.358±
0.011
hyd. 1/24 3/29 4/11 4/30 9/10
n 2 4 8 4 3


D/L VALINE
0.25
0.20
0.15
0.10
0.123±
0.009
hyd. 1/24 3/29 4/11 4/30 9/10
n 2 4 8 3 3

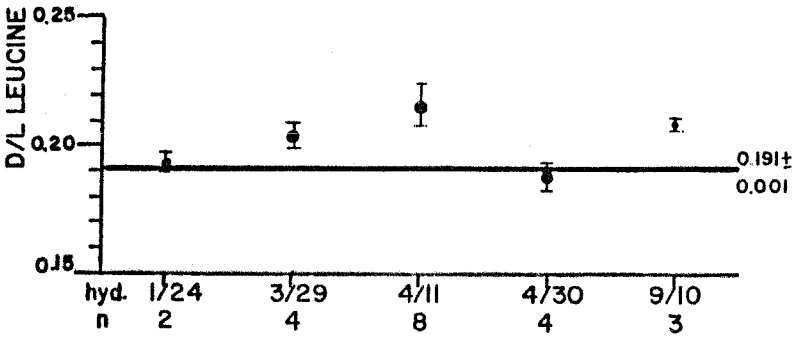
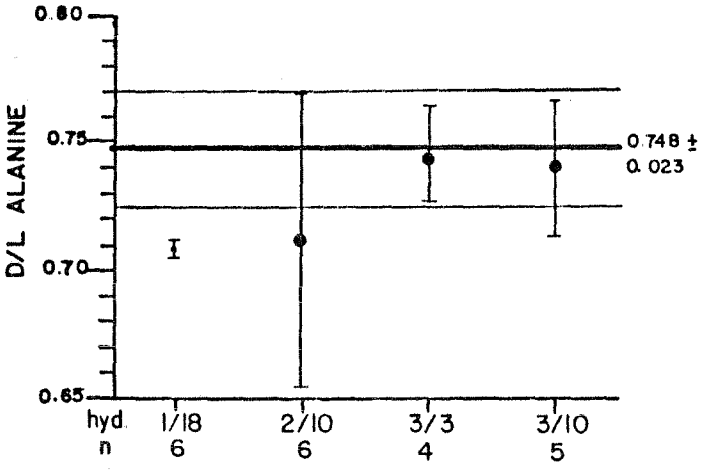
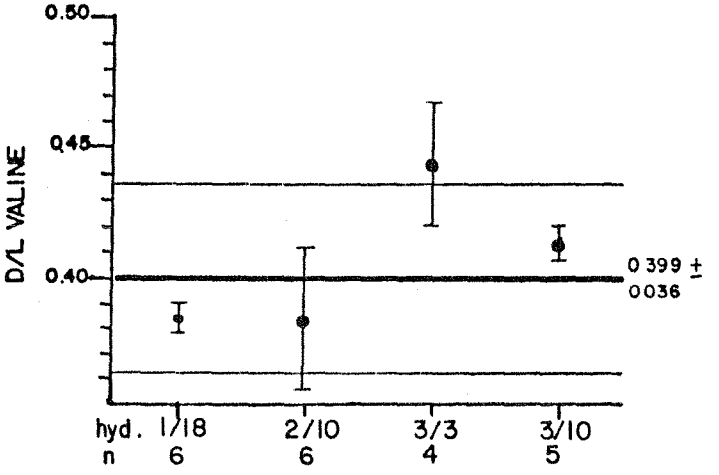
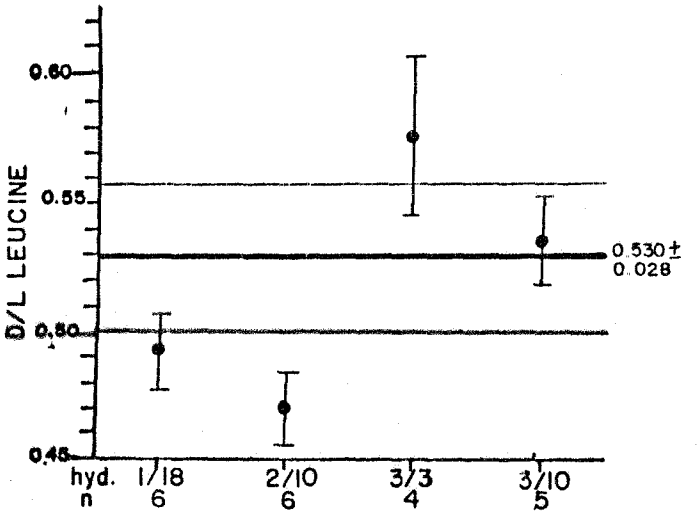

D/L LEUCINE
0.25
0.20
0.15
0.191±
0.001
hyd. 1/24 3/29 4/11 4/30 9/10
n 2 4 8 4 3

Figure 4-2: ILC-B Standard Data. ILC-B standard hydrolyzates of this study are compared to mean D/L values compiled from Univ. of Delaware standards analyzed between January, 1983 through September, 1984. Also included are Univ. of Delaware data summarized in Table 2 of Wehmiller (1984). Standard deviation of each "grand mean D/L value" from the Univ. of Delaware Laboratory is from Wehmiller (1984). Mean D/L values and standard deviations of hydrolyzates (hyd.) of this study are calculated from "n" chromatograms.


0.80
0.75
0.70
0.65
D/L ALANINE
0.748 ±
0.023
hyd.
n
1/18
6
2/10
6
3/3
4
3/10
5


0.50
0.45
0.40
D/L VALINE
0.399 ±
0.036
hyd.
n
1/18
6
2/10
6
3/3
4
3/10
5


0.60
0.55
0.50
0.45
D/L LEUCINE
0.530 ±
0.028
hyd.
n
1/18
6
2/10
6
3/3
4
3/10
5

CHAPTER 5

RESULTS AND DISCUSSION OF FIELD DATA

5.1 Quantification of Data

Raw data consists of three chromatograms per sample. D- and L- amino acid peaks are identified by comparison of peak retention times and magnitudes with positively identified samples. Peaks showing the most reproducible D/L values are alanine, leucine and aspartic acid, similar to peak reproducibility seen in standards samples. Valine, phenylalanine and glutamic acid are less reproducible, as shown by generally higher CVs (App. B and C). D- and L- peaks of phenylalanine and glutamic acid often interfere with each other, resulting in poorer resolution and more variable D/L values. D- and L-proline, and the epimer pair alloisoleucine/isoleucine are disregarded due to their complete lack of resolution on the chromatograms.

Peaks are quantified using three methods. The Hewlett-Packard integrator (model 3390A) calculates both peak height and peak area. These data are compared to peak height data calculated from the chart recorder chromatogram, which is run simultaneously for each sample. It is useful to have all methods available for quantification for the following reasons:

1. Integrator peak area data are more accurate than chart recorder

(peak height) data but,

2. Chart recorder data are necessary for comparison to University of Delaware results obtained prior to 1981, in which only chart recorder peak height data are available.

D/L leucine data vary less than 10% when comparing chart recorder peak height with integrator peak area data determined from hydrolyzates analyzed during this study, as shown by the "% Difference" column in Table 5-1. This is expected since both chromatograms are determined simultaneously during one sample analysis on the GC. However, there are substantial differences when chart recorder data from this study (1984) are compared to chart recorder data from 1982 (Table 2; Wehmiller and Belknap, 1982) for different hydrolyzates (1982 versus 1984) from the same sample valve (Table 5-1). Although chart recorder results are not used for interpretation in this study, the disparity between past and present results make comparison between the two difficult.

Quantification of variability in all methods must be known in order to compare present with existing data. As shown in Table 5-1, D/L leucine values are lower in this data set than those ratios quantified by previous researchers when data from identical methods and valves are compared. This might be explained by a gradual change in capillary column performance.

SAMPLE	INTEGRATOR peak area	INTEGRATOR peak ht.	CHART RECORDER 1982	CHART RECORDER 1984	%DIFF[1]
80-165A	.504	.492	.64	.486	3.6
80-171-1	.204	.204	.270	.185	9.3
80-171-2	.278	.289	.290	.280	0.72
80-171-2B	.238	.271	---	.257	8.0

Table 5-1: Comparison of D/L Leucine Ratios Quantified From Integrator Peak Area and Chart Recorder Peak Height Data. All Integrator Data result from this study. Chart recorder data are obtained from Table 2, Wehmiller and Belknap (1982) and this study (1984). Samples 80-171-2 and -2B are from the same *Mercenaria* valve.

When the column was new, all peaks were resolved within 75min (J.F.Wehmiller, pers.comm.). It now takes up to 120min for complete elution of all peaks. This may result in consistently lower D/L values, even though peaks on the integrator chromatograms do not show broadening or other evidence of poor resolution. Inadequately prepared cation exchange resin used during the desalting step may also account for some variability within this (1984) data set (J.F.Wehmiller, pers.comm., 1984).

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[1] $$\%\text{DIFFERENCE} = \left[\frac{\text{integrator pk. area - chart rec. pk.ht.}}{\text{integrator peak area}}\ 100\right]$$

5.2 Statistical Considerations: Precision

Following the approach described in Wehmiller and Belknap (1982), data are evaluated on four levels: chromatographic, sample, locality and chronostratigraphic precision. Chronostratigraphic precision will be discussed in Chapter 7.

5.2.1 Chromatographic Precision

Chromatographic precision is defined as the variability seen in multiple chromatograms of the same sample. With few exceptions, the CVs for D/L leucine values of both *Mercenaria* and *Anadara* genera never exceed 5% (Apps. B,C). When D/L leucine CVs for each genus are averaged, mean values are 2.76% for *Mercenaria* (23 samples total); and 2.44% for *Anadara* (10 samples total) when all sites are considered. Alanine yields equally reproducible results, with CVs usually ranging from 1 to 4% for *Mercenaria* and 1 to 9% for *Anadara*. Valine, phenylalanine and glutamic acid are less reproducible, often showing CVs exceeding 10% for both genera. Aspartic acid D/L values show CVs usually ranging from 0.2 to approximately 5%. However, this residue is more abundant relative to other amino acids analyzed and large, poorly resolved D- and L- peaks often result. It should also be noted that alanine and leucine D/L values are equally reproducible in samples of all ages. There is no significant trend of higher variability in the D/L values of older samples for these residues.

5.2.2 Sample Precision

Sample precision is defined through multiple analyses of the same shell. This was quantified using *Mercenaria* valves only, as an entire *Anadara* valve is often dissolved for one hydrolyzate. It is noted that D/L leucine CVs for both whole shell and hinge sample *Anadara* hydrolyzates do not exceed 6.0% (App.C).

Hinge and margin samples are compared from the Gomez Pit field site (83GP-89 and -89M). This specimen is extensively leached and chalky and subsequently has yielded D/L leucine values which differed by 80% between hinge and margin. These sample results were not accorded much emphasis in data analysis.

Multiple analyses were also performed on New Light Pit samples, and these are compared to previous analyses of the same shell (Wehmiller and Belknap, 1982; Belknap, 1979). Because so much of sample 80-171-2 has been used, hinge samples could not be taken. Results show a D/L leucine variation of 17% between sub-samples of the same shell (80-171-2 and 80-171-2B, Table 5-1). In addition, present ratios are substantially below those published previously (Wehmiller and Belknap, 1982).

To further quantify intrashell variation, sample 83NB-126 was cut into 5 sections (-126 A through E) and analyzed. All sections show good

chromatographic precision, yet there is considerable variation about a mean D/L leucine value for the shell as a whole (Fig.4-1, App.B-1). Sample 83NB-126 yields a mean D/L leucine value of 0.484 $\pm$0.046, which is somewhat below the mean D/L leucine value of 0.530 for the Norris Bridge site. The disparity between hinge and margin sample D/L values holds true for alanine and valine residues also.

Hinge and margin samples from another valve (83NB-12 and -12M) are also compared (App.B). Again, low CVs are seen for individual samples . Both hinge and margin data are in general agreement with the calculated mean Norris Bridge D/L leucine value.

In conclusion, when considering *Mercenaria* D/L values and CVs, both hinge and margin samples are internally consistent, as seen by low D/L leucine CVs for most field samples (Apps. B, B-1). However, when several subsamples are taken from one *Mercenaria* valve, there is a considerable range of D/L values. A 10% variation in D/L leucine values from subsamples of the same valve is unacceptable (e.g. 83NB-126V through Z). To reduce variation in D/L values seen at a field site, only hinge samples are used.

CVs for D/L leucine values of both whole shell and hinge sample hydrolyzates of *Anadara* are comparable, showing CVs not exceeding 5.7% for all *Anadara* samples (App.C). These data suggest that intrashell variation of

D/L leucine is not substantial. D/L values for alanine and valine are less reproducible, occasionally showing high CVs for both hinge and whole sample hydrolyzates (App.C).

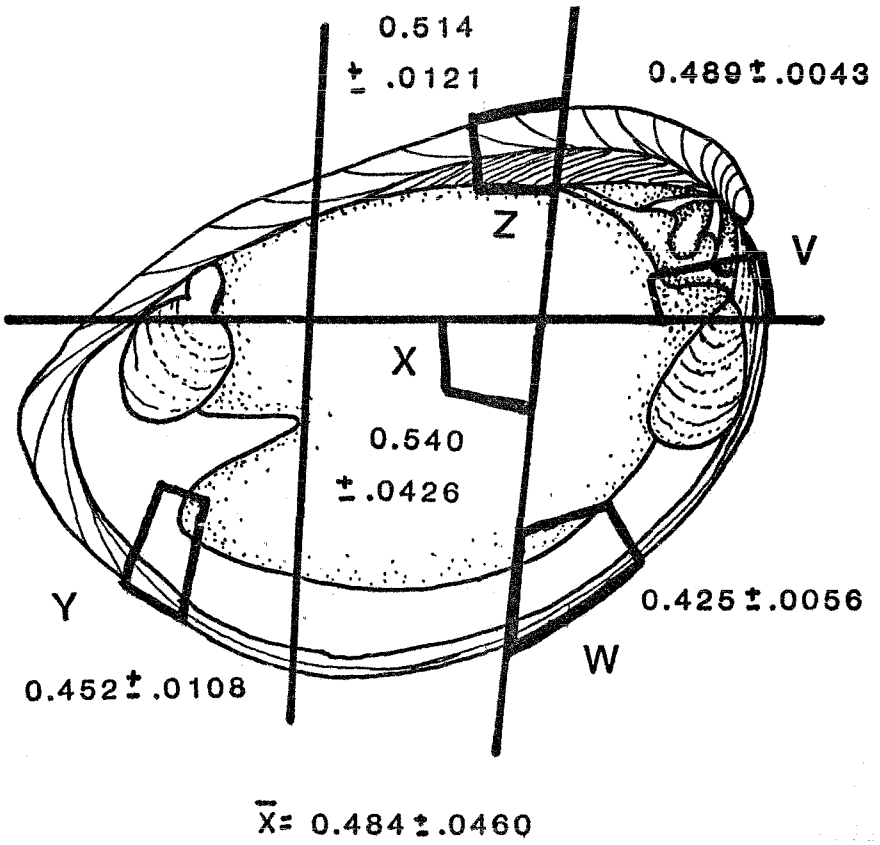


Figure 5-1: D/L Leucine Values of Marginal and Hinge Regions of *Mercenaria.* Sample 83NB-126A through E, from the Norris Bridge field site. Mean D/L Leucine Value = 0.484 ±0.046 for all regions sampled.

5.2.3 Locality Precision

Locality precision is especially significant as it is the basis for geologic interpretation of sample sites. Multiple analyses of several hinge samples are necessary to produce a statistically valid D/L leucine value upon which regional correlation can be based. In this investigation, the Gomez Pit and Norris Bridge sites are sampled and analyzed most extensively, with 6 separate *Mercenaria* at each site. Two *Mercenaria* are reported for both the Moyock Pit and New Light Pit sites. At least 3 *Anadara* samples each are reported for the Gomez Pit, Moyock Pit and Norris Bridge sites.

Mercenaria show the lowest CVs for D/L leucine at all sites. Mean CVs for D/L leucine are 1.88% and 2.65% for analyses of several shells from the Gomez Pit and Norris Bridge sites, respectively. Moyock Pit and New Light Pit samples produced more erratic results, with mean D/L leucine CVs of 5.73% and 18.6%, respectively.

Anadara usually show the lowest CVs for D/L leucine at all field sites. Mean CVs for D/L leucine are as follows: Gomez Pit (1.84%), Norris Bridge (2.86%) and Moyock Pit (2.49%).

In order to determine a "grand mean" D/L leucine value for a locality or defined stratigraphic horizon, and quantify the variability within it, D/L leucine values for each hinge sample (all samples for *Anadara*) analyzed

from that site or horizon are averaged and CVs determined. Table 5-2 shows mean D/L leucine values, standard deviation, CV and number of samples per site for both *Mercenaria* and *Anadara*. This table summarizes data presented in Appendices B and

As shown by Table 5-2, variation of D/L leucine values within any locality does not exceed 12% for either *Mercenaria* or *Anadara*. Each locality shows D/L leucine values that are internally consistent for each genus, as shown by generally low D/L leucine CVs for each site. The discrepancy between *Mercenaria* and *Anadara* D/L leucine values at Moyock Pit will be discussed in Chapter 7.

		MERCENARIA	ANADARA
Gomez Pit	X	0.213	0.265
(Upper strata)	S.D.	0.0109	0.0267
	C.V.%	5.15	9.70
	n	6	3
Norris Bridge	X	0.530	0.515
	S.D.	0.0235	0.0407
	C.V.%	4.43	7.90
	n	6	4
Moyock Pit	X	0.330	0.176
	S.D.	0.0290	0.0207
	C.V.%	8.79	11.8
	n	2	3
New Light Pit	X	0.215	---
(Unit 3)	S.D.	0.0149	---
	C.V.%	6.97	---
	n	3	---

Table 5-2: Mean D/L Leucine Values and CVs for All Field Localities Sampled. "n" represents the number of valves sampled for each genus per site.

CHAPTER 6

INTRA- AND INTERGENERIC RELATIVE RACEMIZATION RATES

6.1 Introduction

To contribute to the understanding of racemization rates in other bivalve genera, amino acid D/L values of g. *Mercenaria* and g. *Anadara* are compared. Results are discussed in terms of apparent relative racemization rates of individual amino acids, and apparent relative racemization kinetics using ASP/LEU values. From this foundation, intergeneric comparisons are made, and the value of *Anadara* for dating purposes is assessed.

There is some question regarding the phyletic level at which different racemization rates can be discerned. There seems to be no single taxonomic level where these rate differences can be detected for all groups of organisms (Lajoie *et al.*, 1980). In studies using mollusks, no species-level differences are found between two species of the Venerid bivalve *Saxidomus*, *S.nuttalli* and *S.giganteus* (Lajoie *et al.*, 1980). Pyrolysis experiments using modern g. *Protothaca* and g. *Mercenaria* shell fragments show that these two bivalves of the Family Veneridae follow similar non-linear kinetic pathways, and these pathways reflect the amino acid compositions found in their fossil analogues

(Keenan, 1983). In the following section, the bivalves g. *Anadara* (Subcl. Pteriomorpha, Superfamily Arcacea) and g. *Mercenaria* (Subcl. Heterodonta, Superfamily Veneracea) are compared.

6.2 Intrageneric Relative Racemization Rates: *Mercenaria*

Mercenaria are found in brackish and marine waters from the intertidal to offshore zones along the entire length of the eastern Atlantic coast of the United States. Two species are often recognized, *M.mercenaria*, extending from the Gulf of St. Lawrence to the Gulf of Mexico; and *M.campechiensis*, ranging from New Jersey south to the Gulf of Mexico (Moore and Teichert, 1971).

It has not been convincingly proven that these two organisms are indeed separate species. In considering the genetic loci responsible for the synthesis of 4 different proteins in both "species" of *Mercenaria*, Pesch (1974) found that a variety of genetic combinations (alleles) is responsible for the production of these proteins in *M.mercenaria* and *M.campechiensis*, indicating a commonality of alleles. Menzel (1969) states that *M.mercenaria* and *M.campechiensis* hybridize readily to the F_2 generation and therefore cannot be considered individual species. One problem in discerning fossil species of any bivalve is their susceptibility to environmentally-induced variation, as reflected in shell morphology. Temperature seems to be the dominant control on growth rate, with turbidity following in importance (Pratt and Campbell,

1974). Morphological variation is displayed in *Mercenaria* collected from the Gomez Pit site. Here, growth lines appear wavy and irregular. This has been interpreted as a result of periodic freshwater influx (Darby, 1983). To simplify the problem of species distinction, characterization of racemization will only be taken to the genus level.

D/L alanine, valine and aspartic acid values are plotted against D/L leucine values for *Mercenaria* (Fig.6-1). Linear regression analysis yields a slope which defines a racemization rate for each amino acid relative to leucine. Both alanine and aspartic acid show significant non-zero y-intercepts, similar to that seen in previous investigation (Lajoie *et al.*, 1980). Aspartic acid consistently shows the greatest positive y-intercept despite good chromatographic resolution, and good correlation of D/L aspartic acid with D/L leucine (r=0.970).

When D/L values for each residue are examined (App.B), the apparent relative racemization rates show the following relationship:

$$\mathbf{ASP} \approx \mathbf{ALA} > \mathbf{LEU} > \mathbf{VAL}$$

Similar apparent relative racemization rates have been reported elsewhere (Keenan, 1983).

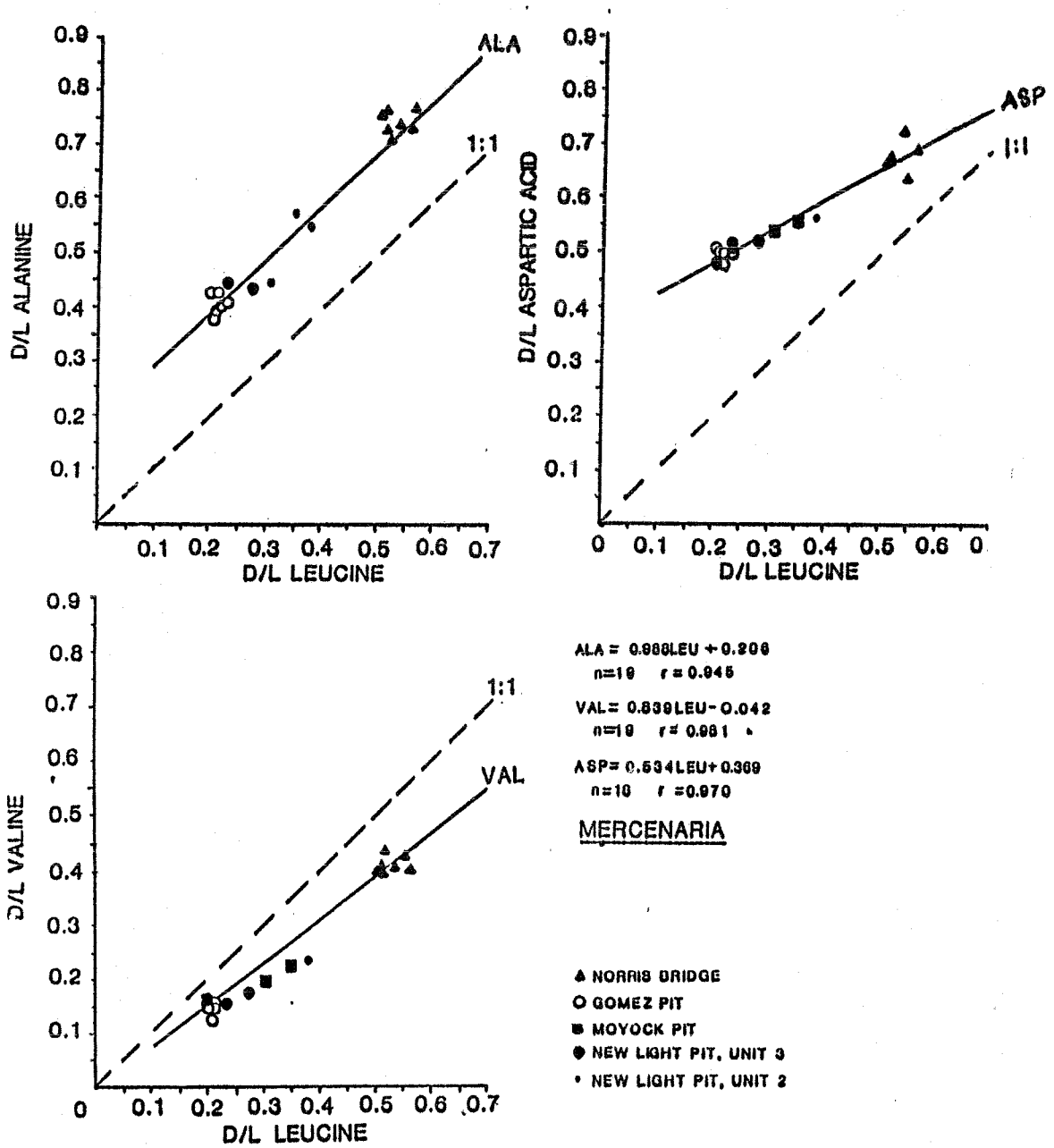


Figure 6-1: Plot Showing Relative Racemization Rates in *Mercenaria*

6.3 Intrageneric Relative Racemization Rates: *Anadara*

Articulated specimens of g. *Anadara* were found coexisting with *Mercenaria* at most sites. The present range of *Anadara* is from North Carolina to the West Indies and Texas (Richards, 1962).

Both alanine and aspartic acid show positive y-intercepts despite good correlation of their respective D/L values with D/L leucine (Fig. 6-2). Comparison of *Anadara* D/L values show the following apparent relative racemization rate relationship:

$$\mathbf{ASP} \approx \mathbf{ALA} > \mathbf{LEU} > \mathbf{VAL}$$

6.4 Intergeneric Comparison Between *Mercenaria* and *Anadara*

Intergeneric comparison of racemization between *Mercenaria* and *Anadara* will be made using the following concepts: Apparent relative racemization rate and apparent relative racemization kinetics.

Intergeneric apparent racemization rate is defined through comparison of the order of observed amino acid D/L values between genera (Lajoie *et al.*, 1980). Intergeneric apparent relative racemization kinetics define the overall pathway of an amino acid D/L value over time, when two genera are compared (Lajoie *et al.*, 1980).

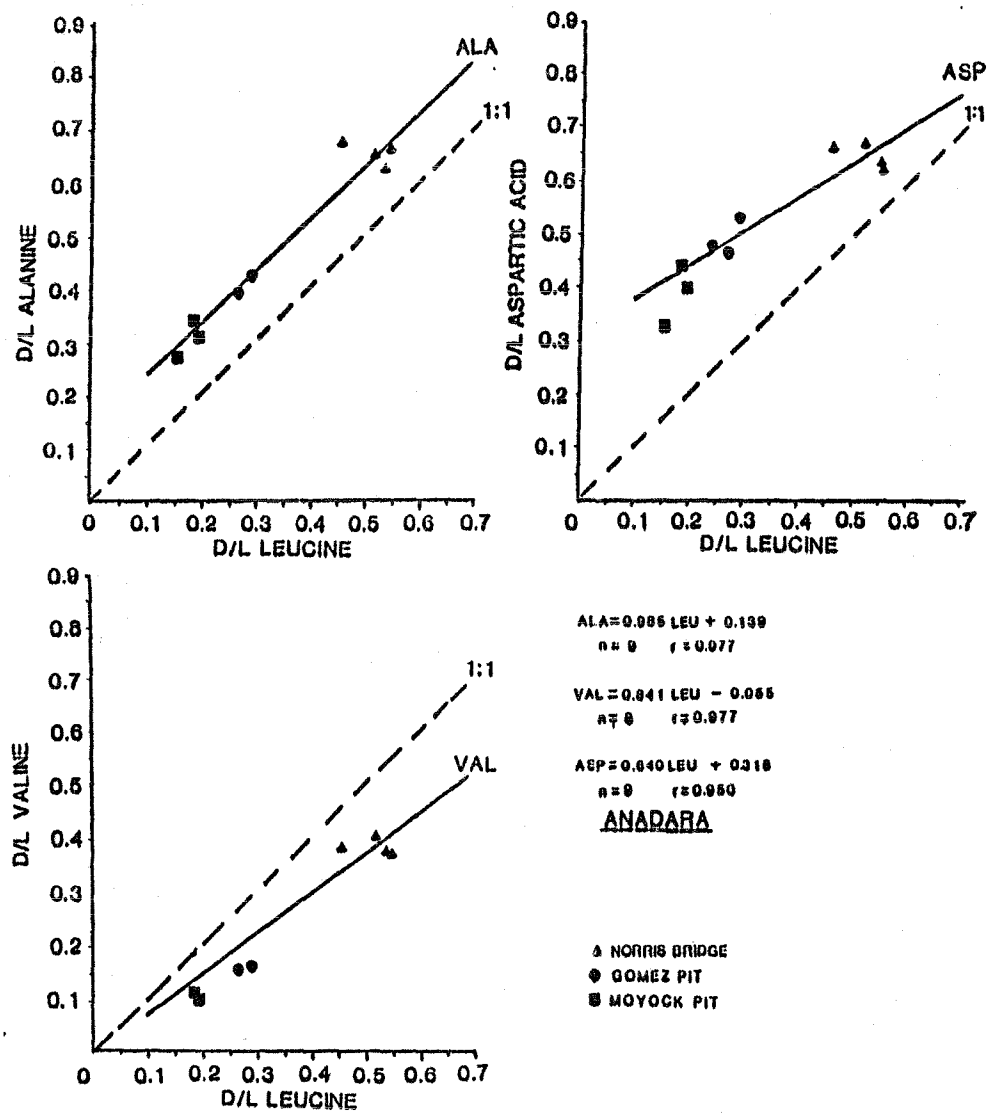


Figure 6-2: Plot Showing Relative Racemization Rates in *Anadara.*

As shown in sections 6.2 and 6.3, *Mercenaria* and *Anadara* show an identical order of enantiomeric ratios. Aspartic acid and alanine are the most extensively racemized amino acids for both genera. When considering Figures 6-1 and 6-2, qualitative comparison of relative racemization rates can be made for *Mercenaria* and *Anadara*. On each plot, the slope of the line defines a relative racemization rate for a particular amino acid D/L value versus D/L leucine. These relative rates (slopes) for D/L alanine, valine and aspartic acid are similar in both *Mercenaria* and *Anadara*.

Qualitative comparison of apparent racemization kinetics between g. *Mercenaria* and g. *Anadara* can be made using aspartic acid/leucine (ASP/LEU) values (Wehmiller, 1980). Aspartic acid is believed to play a significant role in the biomineralization process, as it is the most abundant residue found in the soluble fraction of the organic matrices of fossil and recent *Mercenaria* (Krampitz and Witts, 1979).

Several hypotheses have been proposed to explain the role of aspartic acid in the calcification process. The aspartic acid-rich soluble fraction of the organic matrix may function as a template for the nucleation of $CaCO_3$ crystals. There is a spatial similarity between amino acid residue distances and Ca^{2+}-Ca^{2+} interatomic distances in $CaCO_3$. Negatively-charged aspartic acid residues may bind Ca and permit crystal nucleation (Weiner and Hood, 1975). Alternately, the soluble matrix may selectively bind Ca^{2+} ions, thus regulating $CaCO_3$ crystal nucleation (Wheeler *et al.*,1981; Chave, 1984).

Regardless of the mechanism of calcification in mollusks, aspartic acid is believed to be intimately involved in this process.

The relative abundance of aspartic acid (versus leucine) in mollusk shells has also been related to apparent racemization kinetics (Wehmiller, 1980). Genera having a higher proportion of aspartic acid are categorized as being "slow racemizers", while "fast-racemizing genera" a lower proportion of aspartic acid (Wehmiller, 1980). An increased proportion of aspartic acid is believed to confer stability upon the calcified organic matrix, resulting in slower diagenesis of shell proteins and polypeptides. Early diagenesis of shell proteins in a genus is controlled primarily by the rate of hydrolysis.

Amino acids are more extensively racemized at a terminal position in the protein chain (Smith and Evans, 1980). During early diagenesis, hydrolysis of the protein chain is rapid, resulting in the appearance of extensively racemized terminal and free amino acids. Theoretically, mollusks with higher proportions of aspartic acid are more "stable" with respect to diagenesis, and are thus "slow racemizers". Examples of fast and slow racemizing genera are shown in Table 6-1.

ASP/LEU values for *Mercenaria* and *Anadara* are presented in Appendix C for all field sites. It is difficult to interpret ASP/LEU data for each genus due to the high degree of scatter, even when considering one genus at one particular field site. Table 6-2 summarizes data presented in

Appendix C.

RELATIVE RACEMIZATION RATE	ASP/LEU
"fast" e.g. g.*Busycon*	1-3
"moderate" e.g. g.*Mercenaria*	3-6
"slow" e.g. g.*Crassostrea*	10-20

Table 6-1: Relative Intergeneric Racemization Rates of Selected Modern Mollusks.

LOCALITY	ASP/LEU *Anadara*		ASP/LEU *Mercenaria*	
Gomez Pit	1.4-8.1	X=3.1	1.7-9.6	X=5.3
Moyock Pit	0.6-3.3	X=2.0	2.5-3.7	X=3.1
Norris Bridge	1.9-2.9	X=2.1	1.4-11.4	X=3.8

Table 6-2: ASP/LEU Data for*Mercenaria* and *Anadara* From All Sample Sites.

To conclude, limited *Anadara* data suggest a similarity in racemization behavior to *Mercenaria.* Slopes defining the racemization rate of alanine, valine and aspartic acid (versus leucine) are comparable in both *Mercenaria* and *Anadara.* The relative order of D/L values (apparent relative rate) is identical in both genera. Mean D/L leucine values of *Anadara* and *Mercenaria* are in general agreement for the Gomez Pit and Norris Bridge sites. However, there is a significant difference in D/L leucine values between *Mercenaria* and *Anadara* at the Moyock Pit site. Similar

racemization behavior of these two genera indicate that the D/L leucine values of 0.176 and 0.330 (Table 5-2) represent 2 distinct stratigraphic horizons. More ASP/LEU data are needed to clarify the relationship of racemization behavior between *Mercenaria* and *Anadara*.

CHAPTER 7

AMINOSTRATIGRAPHY: REGIONAL CORRELATION USING D/L LEUCINE VALUES

7.1 Introduction

D/L leucine values have been used previously for correlation of Quaternary deposits of the central Atlantic Coastal Plain (Belknap, 1979; Belknap and Wehmiller, 1980; Wehmiller and Belknap, 1982; McCartan *et al.*, 1982; York, 1984). When considering closely-spaced fossil localities, D/L leucine values can be used to determine age equivalence or estimate difference in age between deposits when D/L values are calibrated using absolute dating methods.

D/L leucine values may also be used for absolute age estimates using the non-linear kinetic model (Wehmiller and Belknap, 1978; Wehmiller, 1981; Wehmiller and Belknap, 1982) and supplemental absolute age and paleoclimate data control. Because the racemization reaction is sensitive to temperature change, temperature history of a fossil must be approximated using an Effective Quaternary Temperature (EQT) to model glacial-to-interglacial climatic change. As the maximum latitudinal range of field sites does not exceed 1° 30", no correction is made for north-to-south climate

variation in samples of equal age.

To model the temperature history of any site in the Norfolk, VA region, the following temperature data are used:

1. A Stage 2 full-glacial temperature reduction ($-\Delta T_{fg}$) of 8° to 12° for 33°N latitude (Wehmiller and Belknap, 1982).

2. A present mean annual air temperature of 16°C for the Norfolk, VA region (p.498; Belknap, 1979).

When 1) and 2) are applied to the square-wave temperature history model (Fig. G-1), glacial-to-interglacial temperature change for the Norfolk, VA region can be estimated.

In this study, different EQTs are calculated for samples having assumed ages of either 75 KA or 125 KA (or greater). Shell samples coincident with the approximate 75 KA U-series coral dates (Apps. E,F) are believed to have endured a greater proportion of cool climate, resulting in a calculated EQT ranging from 8° to 10° for the Norfolk, VA region (App. G). D/L values from these shell samples are interpreted using the 8° EQT curve in the non-linear kinetic model (Fig. 7-1). A 7° $\pm 1^{o}$ EQT has been used to model temperature history for New Light Pit, VA shell samples of 74 ± 4 KA age (Wehmiller and Belknap, 1982).

Shells having an age of 125 KA (Stage 5e) or greater have endured at least one complete glacial-to-interglacial cycle. Calculated EQTs for these shell samples in the Norfolk, VA region range from approximately 10° to 14° (App. G). A 10° EQT is used to estimate amino acid racemization age for these samples. It should be noted that there is accumulating evidence that continental full-glacial temperature reductions in the central and southern Atlantic coastal plain are greater than previously believed (Cronin *et al.*, 1981; Imbrie, Macintyre and Moore, 1983). In the past, climate has been inferred on the basis of marine faunal distributions. However, the terrestrial pollen record suggests cooler climates during the last glacial maximum than is indicated in the marine record (Wehmiller and Belknap, 1982 and references therein). For this reason, lower EQT values are preferred for samples of both 75 KA and 125 KA (or greater) age.

7.2 Age Estimates of Coastal Plain Sites

7.2.1 Gomez Pit, Virginia: Calibration Site

The Gomez Pit site in southeastern Virginia, serves as the calibration site for all subsequent regional age estimates. The 75 $\pm$5 KA U-series coral date (Cronin *et al.*, 1981) is acceptable on the basis of analytical criteria, and D/L leucine values (App.B; Table 5-2) are internally consistent. In addition, the multiple *Mercenaria* horizons sampled from Gomez Pit (App.F) allow for the analysis of the resolving power of the amino acid racemization dating

method.

Previous investigations have modeled temperature histories for the Norfolk, Virginia region (Belknap, 1979; Wehmiller and Belknap, 1982). EQTs of $7 \pm 1^{\circ}$ (Wehmiller and Belknap, 1982) and 11 to 12° (Belknap, 1979) have been used to approximate the temperature history of this region.

In this study, two distinct groups of D/L values have been discerned at Gomez Pit (App. F). These two "aminozones" are believed to represent two distinct age groups. As such, the temperature history for each aminozone is modeled separately. An EQT of 8° is used to interpret D/L data from upper strata of Gomez Pit. Shell samples from these strata are correlated to the 75 ± 5 KA U-series coral date of Cronin *et al.* (1981) (see App. F.). An EQT of 10° is used to interpret D/L data from lower strata of Gomez Pit. Although no U-series coral dates exist for this horizon, *Mercenaria* D/L values suggest an older age.

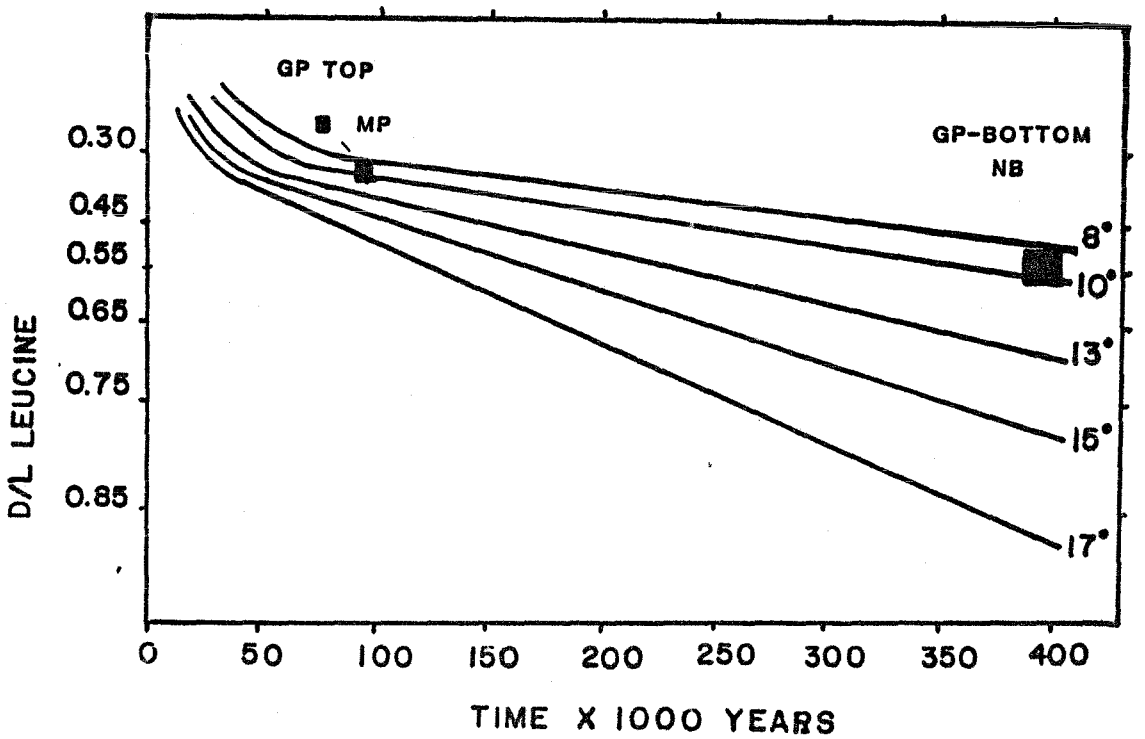


Figure 7-1: Age Estimates Using the Non-Linear Kinetic Model and *Mercenaria* D/L Values. GP=Gomez Pit, MP=Moyock Pit, NB=Norris Bridge.

Mercenaria D/L leucine values from upper strata of the Gomez Pit range from 0.21 to 0.28, corresponding to Stage 5 age when calibrated with the 75 ±5 KA U-series coral date (Cronin *et al.*, 1981). Although *Anadara* D/L leucine ratios are slightly greater than those shown by *Mercenaria* (Table 5-2), these data are consistent with a late Stage 5 age estimate for the upper strata of Gomez Pit. The D/L leucine value of 0.529 seen at the bottom of Gomez Pit (App.F) corresponds to an approximate 400 KA age (Fig. 7-1).

A hiatus within the Sedgefield Member of the Tabb Formation is shown by the gap in D/L values between approximately 0.25 and 0.53 (Fig. 7-2). This difference between mean D/L leucine values of upper and lower strata at the Gomez Pit (0.213 and 0.529, respectively) apparently represents up to 300 KA (Fig. 7-1). The existence of a hiatus *within* the Sedgefield Member is in disagreement with a previous interpretation based on lithology. The Sedgefield Member section in Gomez Pit is interpreted as one "transgressive package", extending upsection from a basal, pebbly to cobbly, fine to coarse sand (Peebles *et al.*, 1984). This "cobble layer" marks the unconformable contact between the Chowan River Formation and Sedgefield Member of the Tabb Formation (Fig. 3-4).

It is possible that early and late Stage 5 is represented in the southwest wall exposure in Gomez Pit (Fig. 7-2). As mentioned previously, *Mercenaria* D/L leucine data from upper strata at this exposure (mean D/L

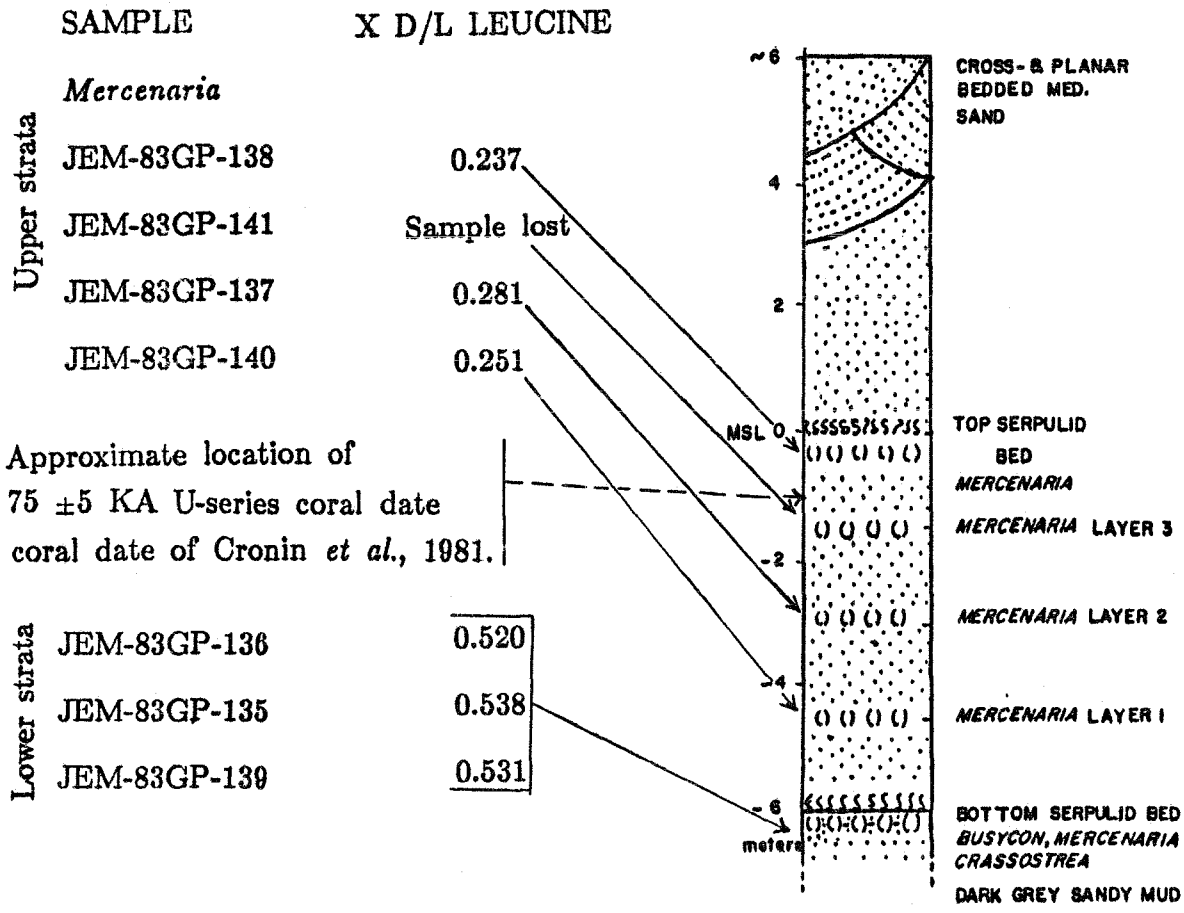


Figure 7-2: Stratigraphic Section from Gomez Pit, Southwest Wall. All D/L leucine values shown are from *Mercenaria* samples.

value = 0.213) are correlated with a 75 ±5 KA U-series coral date (Cronin *et al.*, 1981). *Mercenaria* D/L leucine values from upper strata in Gomez Pit range from 0.21 to 0.28 (Fig. 7-2). Amino acid racemization dating of early and late Stage 5 mollusk samples has been performed at two other sites: Two terraces at Point Loma, near San Diego, CA (Wehmiller *et al.*, 1977); and Fjosanger, western Norway (Miller *et al.*, 1983).

When comparing these three Stage 5 sites on a qualitative basis, the following D/L values (or ranges of values) are found for late and early Stage 5, respectively. Gomez Pit, VA (0.21 to 0.28); San Diego, CA (0.38 and 0.47), and Fjosanger, Norway (0.22 to 0.27). A similar increment of change between late and early Stage 5 D/L values is seen for all sites, suggesting that Gomez Pit may also show late and early Stage 5 age mollusks within the upper strata. More analyses are necessary to clarify this relationship.

7.2.2 Norris Bridge, Virginia

The Norris Bridge locality shows mean D/L leucine values for *Mercenaria* and *Anadara* of 0.51 and 0.53 respectively (Table 5-2). These correspond to an approximate 400 KA age (Fig. 7-1). This 400 KA age is in direct conflict with the 187 ±20 KA U-series coral age obtained from this site (Cronin *et al.*, 1981) . As mentioned in the field site description for Norris Bridge (Chapter 3.2.3), the U-series coral date of 187 KA is suspect on the basis of questionable isotopic ratios and is afforded little consequence in these

interpretations.

This 400 KA age estimate is lower than the previously published age estimate of 700 to 800 KA (Wehmiller and Belknap, 1982). As discussed previously, ILC-B leucine values vary to 11% from University of Delaware mean D/L leucine values for the ILC-B standard. Despite the difference between ILC-B standard data, comparison of D/L leucine values between Norris Bridge and Gomez Pit determined in this study is consistent with previous work and supports previous estimates of the difference in age between these two sites.

7.2.3 New Light Pit, Virginia

Although New Light Pit is now flooded, existing samples have been analyzed to correlate deposits with those at Gomez Pit, 1 km to the south.

Two groups of *Mercenaria* D/L leucine values have been reported previously from this site (Belknap, 1979). Two groups are seen in data from this study. New Light Pit Units 3 and 2 (App.F) show mean D/L leucine values of 0.215 and 0.380, respectively. The 0.215 *Mercenaria* D/L leucine value from the upper Unit 3 and the U-series coral date of 74 $\pm$4 KA (Cronin *et al.*, 1981) are consistent with D/L leucine values and the U-series coral date found in the upper strata in Gomez Pit. A hiatus in D/L leucine values (0.22 to 0.38) is seen between samples from Unit 3 and Unit 2,

respectively (App. F). Duration of this apparent hiatus (<200 KA), as approximated from amino acid racemization age estimates, is not as great as that seen in Gomez Pit. The D/L leucine value of 0.380 for this Unit 2 sample (App. B, pg. 92) is lower than the 0.422 D/L leucine value determined from previous analysis of the same valve (pg.526; Belknap, 1979). Because so much of this shell has been used, a hinge sample was not available. On the basis of discussion in Chapter 5.2.2, this sample may be suspect because the hinge region was not used for analysis. No *Anadara* valves were analyzed from this site.

7.3 Moyock Pit, North Carolina

Spoil piles from Moyock Pit have yielded a *Mercenaria* mean D/L leucine value of 0.330, corresponding to an approximate age of 100 KA. *Anadara* show a mean D/L leucine value of 0.18. Assuming that these two genera show similar racemizing behavior (Chapter 6), there is evidence for the existence of several molluscan horizons, similar to those seen in Gomez Pit and New Light Pit.

The *Anadara* mean D/L leucine value (0.18) is consistent with Gomez Pit D/L leucine values (0.21 to 0.28) calibrated with the 75 $\pm$5 KA U-series coral date (Cronin *et al.*, 1981). The *Mercenaria* mean D/L leucine value (0.330) is not consistent with the Gomez Pit calibration.

A 72 ±4 KA U-series coral date has been collected from a spoil pile at the Moyock Pit locality (Cronin *et al.*, 1981). This U-series coral date is tentatively correlated with the 0.18 *Anadara* D/L leucine value, using Gomez Pit amino acid calibration as a guide.

The 10° EQT curve is used to interpret the mean *Mercenaria* D/L leucine value of 0.330 (Fig. 7-1). This 0.330 value is assumed to be somewhat older than 75 KA, and is interpreted using the EQT of a 125 KA sample. The use of a 10° EQT results in an age estimate of 100 KA for these *Mercenaria* samples. This age estimate is considered tentative for the following reasons:

1. Complete lack of stratigraphic or absolute age control.

2. The D/L value of 0.330 falls at or near the transition zone of the non-linear kinetic curve. There is a complex sequence of reactions characterizing this region of the curve. At present, it is difficult to interpret D/L values in this region of the curve without absolute age control.

CHAPTER 8

SUMMARY AND CONCLUSIONS

The three goals of this study are as follows:

1. To show reproducibility of results of the amino acid racemization dating method on several levels for both *Mercenaria* and *Anadara*.

2. To correlate the closely spaced field sites of this study using these *Mercenaria* and *Anadara* D/L data.

3. To propose age estimates for these field sites by applying amino acid data to the non-linear kinetic model of Wehmiller and Belknap (1982).

Reproducibility of results is shown through statistical analysis of both standard and field samples. Several ILC-A and ILC-B standard samples were run throughout this study to quantify variation resulting from sample preparation and system operation. D/L alanine and leucine are found to be most reproducible. Standard samples show good chromatographic precision. D/L leucine CVs of multiple chromatograms of any ILC-A or -B hydrolyzate do not exceed 5.26% (App. A). When D/L leucine values from all JEM

standard hydrolyzates of either ILC-A or ILC-B are averaged, CVs of 5.7% (5 samples) and 9.1% (4 samples) respectively, are shown indicating relatively good sample precision. Accuracy of standards results is quantified through comparison of standard data personally analyzed with data from other Univ. of Delaware laboratory analysts. Relative error between these two data sets is commonly under 5% when identical methods are compared, though some significant deviations (up to 11%) have been noted (see p.36).

D/L data from field samples are also interpreted on several levels to determine reproducibility of results within a particular hydrolyzate (chromatographic precision); within one mollusk valve (sample precision); and from several valve or hinge samples from one site (locality precision). Chromatographic precision is generally reproducible to within 5% for D/L leucine in both *Mercenaria* and *Anadara*. Intrashell variation is found to exceed 10% for multiple *Mercenaria* shell sub-samples. For this reason, only hinge samples of larger bivalves are used for analysis. Low CVs (<6.0%) for D/L leucine in both hinge and whole shell *Anadara* hydrolyzates suggest that intrashell variation of D/L leucine is not as significant in this genus.

Reproducible results are found when analyzing several hinge or shell samples from any one field site. D/L leucine CVs for *Mercenaria* are as follows: Gomez Pit (5.15%); Norris Bridge (4.43%); Moyock Pit (8.79%) and New Light Pit (6.97%). D/L leucine CVs for *Anadara* are comparable: Gomez Pit (9.70%); Norris Bridge (7.90%) and Moyock Pit (11.8%).

Intra- and intergeneric relative racemization rates were investigated to establish a qualitative relationship between *Mercenaria* and *Anadara*. Both genera show identical apparent relative racemization rates (i.e. ASP$\geq$ALA>LEU>VAL). Both genera also show similar relative racemization rates of alanine, valine and aspartic acid (relative to leucine) as shown by Figures 6-1 and 6-2. Comparable slopes of lines in these plots suggest similar racemization behavior.

With good precision and accuracy of standards data, and acceptable reproducibility of field data, D/L leucine values may be used for correlation of closely spaced units. Four groups of D/L values (or "aminozones") are proposed for correlation of field sites of this study (Fig. 8-1). As mentioned previously (p.62), there is a hiatus shown by a gap in D/L values within the Sedgefield Member of the Tabb Formation in Gomez Pit. This hiatus occurs between aminozone I ("upper strata", D/L leucine ranges from 0.21 to 0.28) and aminozone IV ("lower strata", D/L leucine equals 0.53). This hiatus is not recognized in lithologic study at the Gomez Pit site (Peebles *et al.*, 1984).

A hiatus is also shown by a gap in D/L values at the New Light Pit site, 1 km to the north of Gomez Pit. Although aminozone I is found at both sites, *Mercenaria* samples obtained below the aminostratigraphic hiatus at New Light Pit show a mean D/L leucine value of 0.38 (aminozone III). Belknap (p.465; 1979) reports an aminostratigraphic hiatus of similar magnitude at the New Light Pit (between units 2 and 3; see App. F, this

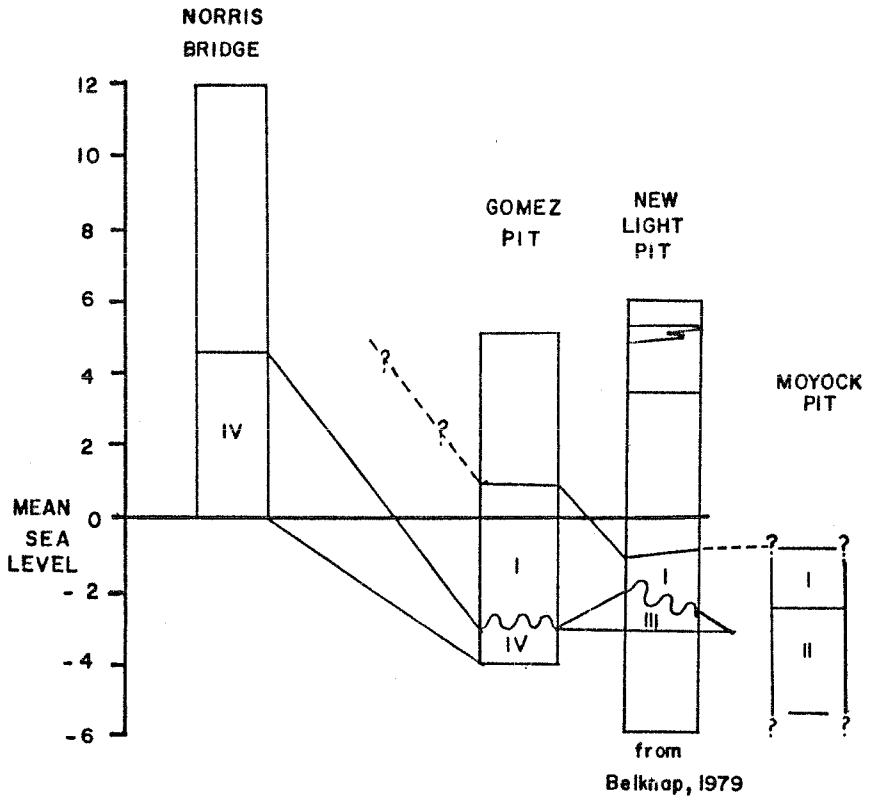
thesis).

Mercenaria and *Anadara* obtained from the Norris Bridge site show D/L leucine values corresponding exclusively to aminozone IV. This study shows Norris Bridge *Mercenaria* D/L values as consistently lower than previous analyses (Wehmiller and Belknap, 1982). However, when comparing D/L values between sites, Norris Bridge and New Light Pit show similar relative ages (Table 8-1).

	Norris Bridge	New Light Pit, Unit 3
W. and B. (1982)	0.623 ±0.015	0.277 ±0.012
This study	0.530 ±0.024	0.215 ±0.015

Table 8-1: Comparison of *Mercenaria* D/L Data from Gomez Pit and Norris Bridge. The difference in D/L values between these two sites is similar: 0.346 (Wehmiller and Belknap, 1982) and 0.315 (this study).

This relative difference in D/L values between Norris Bridge and New Light Pit is consistent with previous age estimates of the Norris Bridge site (Wehmiller and Belknap, 1982). *Mercenaria* and *Anadara* D/L values suggest that the Norris Bridge site is considerably older than the New Light Pit site. The 0.530 D/L leucine value for Norris Bridge is not consistent with the 187 ±20 KA U-series coral date for this site (Cronin *et al.*, 1981). The 0.530 D/L leucine value suggests an age of 400 KA when a 10° EQT curve is used in the non-linear model (Fig. 7-1).



AMINOZONES

	MERCENARIA	*ANADARA*
I	0.21-0.28 ± 0.01	0.18 ± 0.02
II	0.33 ± 0.03	
III	0.38 ± 0.002	
IV	0.53 ± 0.02	0.52 ± 0.04

Figure 8-1: Aminostratigraphic Framework for Some Coastal Plain Sites, Southeastern Virginia and Northeastern North Carolina. Sea level data from Cronin *et al.* (1981) and Belknap (1979). Aminozones I and IV at Gomez Pit correspond to the Sedgefield Member of the Tabb Formation of Peebles *et al.* (1984).

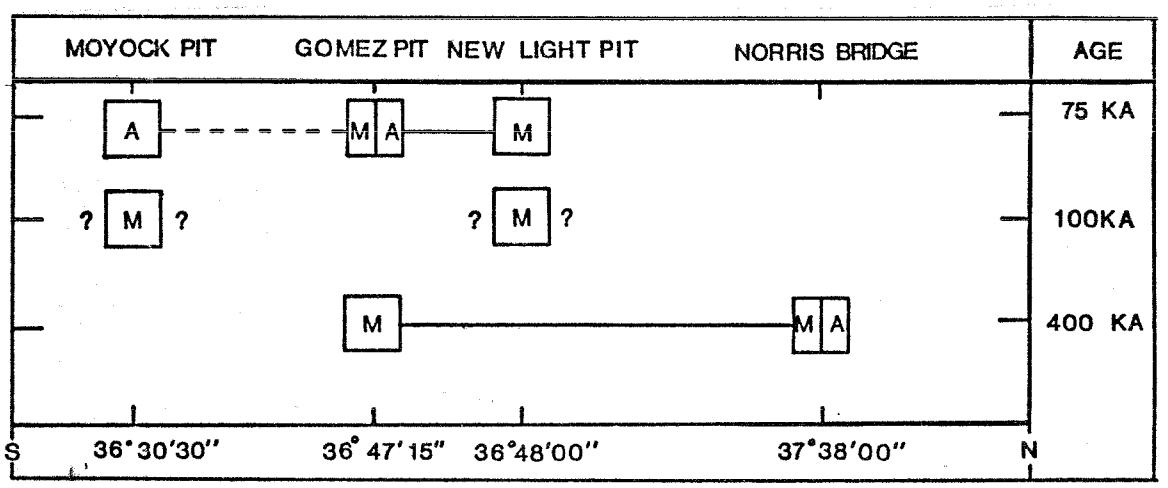


Figure 8-2: Tentative Correlation of Field Sites of This Study. Ages are estimated using g. *Anadara* (A) and g. *Mercenaria* (M) D/L values applied to the non-linear kinetic model.

Fig. 8-2 shows correlation of field sites based on age estimates using the non-linear kinetic model. *Mercenaria* having D/L values of 0.33 (Moyock Pit) and 0.38 (New Light Pit) are tentatively assigned an age ranging between 100 and 200 KA. The Moyock Pit locality presents an unusual problem. Two aminozones exist at this site (I, 0.18 and II, 0.33). Assuming that *Anadara* and *Mercenaria* show similar racemization behavior, there is evidence of two stratigraphic horizons. The stratigraphic location of the 72 ± 4 KA U-series coral date from this site is unknown. Using the data from Gomez Pit as a guide, the 0.18 *Anadara* mean D/L leucine value probably corresponds with the 72 ± 4 KA U-series coral date (Cronin *et al.*, 1981). A tentative age assignment of 100 KA is made when the 10° EQT is used to interpret the mean *Mercenaria* D/L leucine value (0.330) from the Moyock Pit site. Further analyses of Moyock Pit *Mercenaria* are necessary to elucidate the following stratigraphic and kinetic relationships:

1. The possible existence of several *Mercenaria* horizons to account for the disparity between *Mercenaria* and *Anadara* D/L leucine values at this site.

2. The calibration of a "transition zone" on the non-linear kinetic curve. With D/L leucine values ranging from 0.30 to 0.38, these samples fall near the slope break on the non-linear kinetic curve for a 10° EQT. Further amino acid analyses and absolute dating of samples from this locality will calibrate this poorly understood region of the curve.

There are two major aminostratigraphic problems which need to be resolved through further analyses.

1. The significance of the Moyock Pit site. A described stratigraphic section at this site to determine the existence and number of *Mercenaria* horizons is needed. U-series dating of corals coexisting with mollusks (showing a 0.33 D/L leucine value) will calibrate the non-linear region of the kinetic model.

2. The existence of late and early Stage 5 mollusks at the Gomez Pit site. Further analyses of all *Mercenaria* layers (App. F) are needed to confirm a late and early Stage 5 age correlation with D/L values ranging from 0.21 through 0.28, respectively.

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APPENDIX A

ILC STANDARDS DATA

A.1 Precision of Standard Analyses

Shown are mean values (X), standard deviations (S.D.), coefficients of variation (C.V.) and % relative error (% R.E.) for all ILC-A and ILC-B samples run by the author and other University of Delaware analysts during this study. % Relative error is the difference between D/L data from this study and mean D/L data determined from other UD-ILC-A and ILC-B powder standards prepared by the isopropanol method. University of Delaware data shown in this appendix are compiled from chromatograms run between January, 1983 and September, 1984. Also included are University of Delaware mean D/L values previously published (Lab E, Table 2; Wehmiller, 1984). Wehmiller (1984) data summarizes ILC samples analyzed between January, 1982 and October, 1982.

JEM-ILC-A1/24: 2 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
X	0.341	0.124	0.194	--	0.185	0.137
S.D.	0.0184	0.005	0.0021	--	0.0177	0.0311
C.V%	5.29	4.03	1.08	--	9.57*	22.7
n	2	2	2	--	2	2
%R.E.	4.7	0.81	1.57	--		

JEM-ILC-A3/29: 4 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
X	0.375	0.137	0.204	0.381	0.237	0.162
S.D.	0.0134	0.0090	0.0046	0.0438	0.0087	0.0392
C.V/%	3.57	6.57	2.25	11.5	3.67	24.2
n	4	4	4	2	4	4
%R.E.	4.7	11.4	6.8			

JEM-ILC-A4/11: 8 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
X	0.368	0.138	0.216	0.348	0.236	0.14
S.D.	0.005	0.0034	0.0068	0.014	0.017	0.025
C.V.%	1.36	2.47	3.1	4.02	7.2	17.9
n	8	8	8	4	8	8
%R.E.	2.8	12.2	12.3			

JEM-ILC-A4/30: 4 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
X	0.397	0.123	0.187	0.368	0.227	0.165
S.D.	0.0532	0.003	0.0062	0.0018	0.234	0.0048
C.V.%	13.4	0.81	3.3	0.49	10.3	2.9
n	4	3	4	4	4	4
%R.E.	10.9	0	2.1			

JEM-ILC-A7/18: 6 chromatograms. Data not analyzed in time for inclusion in the statistical analysis section.

	ALA	VAL	LEU	ASP	PHE	GLU
X	.243	0.077	0.117	0.467	---	0.142
S.D.	0.047	0.009	0.004	0.034	---	0.001
C.V.%	19.5	11.4	3.00			
n	6	5	6	5	1	3
%R.E.	32.1	37.4	38.7			

JEM-ILC-A9/10: 3 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
X	0.372	0.137	0.209	0.354	0.243	0.164
S.D.	0.012	0.002	0.001	0.001	0.026	0
C.V.%	3.27	1.10	5.26			
n	3	3	3	3	3	1
R.E.%	3.9	11.4	9.4			

JEM-ILC-B1/18: 6 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
X	0.709	0.384	0.493	0.702	0.542	0.355
S.D.	0.0014	0.0054	0.0145	0.0041	0.0386	0.033
C.V.%	0.19	1.41	2.94			
n	6	6	6	6	5	6
%R.E.	5.2	3.8	7.0			

JEM-ILC-B2/10: 7 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
X	0.713	0.383	0.470	0.668	0.486	0.353
S.D.	0.0573	0.283	0.0148	0.0639	0.0271	0.127
C.V.%	8.04	7.39	3.15			
n	6	6	6	6	6	5
%R.E.	4.7	4.0	11.3			

JEM-ILC-B3/3: 4 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
X	0.744	0.444	0.576	0.704	0.594	0.361
S.D.	0.0184	0.0243	0.0292	0.0174	0.0447	0.0411
C.V.%	2.47	5.47	5.07			
n	4	4	4	4	4	4
%R.E.	0.54	11.2	8.0			

JEM-ILC-B3/10: 5 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
X	0.740	0.413	0.535	0.698	0.570	0.379
S.D.	0.0262	0.0066	0.0173	0.0053	0.0063	0.0041
C.V.%	3.54	1.60	3.23			
n	5	5	5	5	4	5
%R.E.	1.1	3.5	0.9			

LLY-ILC-A1/83: 3 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.373	0.115	0.18	0.243	0.228	0.191
S.D.	0.045	0.015	0.017	0.193	0.007	0.019
C.V.%	12.3	12.9	9.6	79.4	3.04	9.95
n	3	3	3	2	3	3

JFW-ILC-A7/7/83: 2 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.352	---	0.197	0.362	0.231	0.159
S.D.	0.006	---	0.001	0.008	0.002	0.008
C.V.%	1.61	---	0.51	2.21	0.86	5.34
n	2	0	2	2	2	2

DS-ILC-A11/83: 3 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.393	0.118	0.194	0.348	0.284	0.149
S.D.	0.029	0.008	0.005	0.008	0.065	0.013
C.V.%	7.57	6.78	2.58	2.58	2.29	22.9
n	3	3	3	2	2	2

JFW-ILC-A1/4/84: 5 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.349	0.126	0.199	0.344	0.240	0.157
S.D.	0.006	0.003	0.006	0.027	0.01	0.013
C.V.%	1.72	2.38	3.02	7.85	4.17	8.28
n	5	5	5	3	4	4

JFW-ILC-A1/10/84: 4 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.345	0.121	0.186	0.339	0.233	0.145
S.D	0.006	0.012	0.003	0.016	0.009	0.011
C.V.%	1.74	9.92	1.61	4.72	3.86	7.59
n	4	4	4	4	4	3

JFW-ILC-A7/11/84: 2 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.350	0.126	0.191	--	0.212	0.158
S.D.	0.007	0.002	0.001	--	0.003	0.01
C.V.%	2.00	1.59	0.52	--	1.41	6.33
n	2	2	2	--	2	2

JFW-ILC-A9/12/84: 3 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.336	0.116	0.178	0.367	0.186	0.152
S.D.	0.004	0.004	0.003	---	0.004	0.009
C.V.%	1.19	3.45	1.68	---	2.15	5.92
n	3	3	3	1	3	3

JFW-ILC-B1/83: 5 chromatograms (Noisy baseline)

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.737	0.339	0.473	0.714	0.549	0.374
S.D.	0.101	0.037	0.011	0.040	0.041	0.044
C.V.%	13.7	10.9	2.32	5.60	7.47	11.8
n	4	5	5	4	4	5

JFW-ILC-B2/83: 5 chromatograms (Noisy baseline)

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.689	0.395	0.558	0.706	0.653	0.393
S.D.	0.128	0.041	0.048	0.037	0.058	0.031
C.V.%	18.6	10.4	8.60	5.24	8.88	7.89
n	5	5	5	3	4	5

JFW-ILC-B7/83: 5 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.765	0.361	0.507	0.668	0.579	0.378
S.D.	0.031	0.032	0.018	0.055	0.006	0.023
C.V.%	4.05	8.86	3.55	8.23	1.13	6.08
n	5	5	5	5	3	4

JFW-ILC-B8/83: 5 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.762	0.425	0.588	0.671	0.610	0.363
S.D.	0.030	0.028	0.030	0.037	0.025	0.020
C.V.%	3.93	6.59	5.10	5.51	4.09	5.51
n	5	4	5	5	5	5

JFW-ILC-B9/30/83: 4 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.722	0.368	0.524	0.673	0.574	0.394
S.D.	0.033	0.02	0.022	0.034	0.014	0.023
C.V%	4.22	5.43	4.2	5.05	2.44	5.84
n	4	4	4	4	4	4

JFW-ILC-B2/22/84: 5 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.714	0.380	0.467	0.684	0.536	0.347
S.D.	0.015	0.023	0.015	0.01	0.031	0.029
C.V.%	2.10	6.05	3.21	1.46	5.78	8.36
n	4	4	5	5	5	5

JFW-ILC-B3/12/84: 3 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.722	0.399	0.503	0.698	0.559	0.378
S.D.	0.021	0.003	0.002	0.014	0.021	0.018
C.V.%	2.91	0.75	0.397	2.01	3.76	4.76
n	3	3	3	3	3	3

JFW-ILC-B4/19/84: 6 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.777	0.464	0.610	0.725	0.601	0.400
S.D.	0.008	0.013	0.005	0.005	0.03	0.340
C.V.%	1.03	2.80	0.82	0.69	4.99	8.50
n	6	6	6	6	6	6

LLY-ILC-B5/2/84: 4 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.736	0.388	0.506	0.702	0.537	0.350
S.D.	0.04	0.004	0.009	0.007	0.01	0.016
C.V.%	5.43	1.03	1.78	0.997	1.86	4.57
n	4	4	4	4	4	4

JFW-ILC-B7/17/84: 2 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.795	0.421	0.554	0.678	0.563	0.380
S.D.	0.103	0.022	0.032	0.045	0.022	0.012
C.V.%	13.0	5.23	5.78	6.67	3.91	3.16
n	2	2	2	2	2	2

JFW-ILC-B-7/27/84: 3 chromatograms

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.735	0.420	0.538	0.715	0.562	0.360
S.D.	0.009	0.007	0.002	0.003	0.01	0.01
C.V.%	1.22	1.67	0.37	0.42	1.78	2.78
n	3	3	3	3	3	3

ILC-A: The following summarizes ILC-A powder standard samples run between January, 1982 and October, 1982. Taken from Lab E, Table 2; Wehmiller (1984).

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.365	0.136	0.202	0.379	0.238	0.187
S.D.	0.0011	0.009	0.001	0.005	0.027	0.008
C.V.%	1.92	6.6	0.49	1.32	11.3	4.28

ILC-B: The following table summarizes ILC-B powder standard samples run at the UD lab between January, 1982 and October, 1982. These data summarized as Lab E, in Table 2 of Wehmiller (1984).

	ALA	VAL	LEU	ASP	PHE	GLU
D/L	0.770	0.429	0.535	0.712	0.611	0.415
S.D.	0.023	0.037	0.028	0.014	0.029	0.023
C.V.%	2.99	8.62	5.23	1.97	4.65	5.54

A.2 Accuracy of Standard Data

As discussed in Chapter 4.3, JEM-ILC-A and -B data are compared to University of Delaware Laboratory standard data of other analysts (D. Sirkis, J.F. Wehmiller, L.L.York, unpub. data, 1983, 1984). These UD laboratory data also include published D/L values (Wehmiller, 1984) summarizing analyses between January, 1982 and October, 1982.

Mean D/L values, standard deviations (S.D.), coefficients of variation (C.V.%) and number of chromatograms (n) are shown for both data sets. "n" does not include those chromatograms summarized in Wehmiller (1984). Both Tables A-1 and A-2 are compiled from data presented in Appendix A.1.

JEM-ILC-A	ALA	VAL	LEU
X	0.371	0.132	0.202
S.D.	0.020	0.008	0.011
C.V.%	5.4	6.1	5.7
n	21	21	21

JEM-ILC-B	ALA	VAL	LEU
X	0.727	0.406	0.519
S.D.	0.018	0.029	0.047
C.V.%	2.5	7.1	9.0
n	22	22	22

Table A-1: Statistical Data for ILC-A and ILC-B Standards Analyzed by June Mirecki, during the period January through July, 1984. ILC-A consists of 5 hydrolyzates; ILC-B consists of 4 hydrolyzates.
These data are summarized in Table 4-2 as "B"

UD-ILC-A	ALA	VAL	LEU
X	0.358	0.123	0.191
S.D.	0.018	0.007	0.009
C.V.%	5.11	5.69	4.71
n	22	20	22

UD-ILC-B	ALA	VAL	LEU
X	0.748	0.399	0.530
S.D.	0.031	0.035	0.043
C.V.%	4.14	8.77	8.11
n	45	45	47

Table A-2: Statistical Data for ILC-A and ILC-B Standards Run By Other Analysts in the Univ. of Delaware Laboratory (D.Sirkis, J.F. Wehmiller, L.L. York, unpub.data; 1983,1984; Wehmiller, 1984). These data are summarized in Table 4-2, as "A".

APPENDIX B

STATISTICAL ANALYSIS OF FIELD DATA: *MERCENARIA*

Gomez Pit

All samples analyzed, with the exceptions of JEM-83NB-126V through Y, are hinge samples. Those samples designated with the suffix "-M" are valve margin samples. "n" represents the number of chromatograms used to determine the mean residue D/L value for each sample.

JEM-83GP-93

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.397	.146	.217	.479	.196	.134	Central
S.D.	.012	.002	.004	.008	.02	.02	
C.V%	3.03	1.37	1.84	1.67	10.2	14.8	
n	4	4	4	3	3	2	

JEM-83GP-95

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.387	.126	.210	.483	.263	.155	Central
S.D.	.012	.006	.001	.001	.081	.007	
C.V.%	5.43	4.80	.478	.207	30.8	4.52	
n	4	4	4	2	4	4	

JEM-83GP-75

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.376	.145	.203	.496	.263	.122	4'level
S.D.	.013	.013	.007	.05	.018	.025	
C.V.%	3.46	8.97	3.45	10.1	6.72	20.9	
n	3	3	3	2	2	2	

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JEM-83GP-89M Margin sample

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.409	.144	.231	.496	.194	.181	7.5' leve
S.D.	.009	.02	.04	.035	.036	0	
C.V.%	2.21	13.9	1.74	7.07	18.6	0	
n	2	2	2	2	2	1	

JEM-83GP-89 Data disregarded in locality precision calculation due to conflict with D/L leucine in sample JEM-83GP-89M.

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.510	.206	.415	.582	.454	.223	7.5' leve
S.D.	.003	.003	.014	.027	.078	.054	
C.V.%	.59	1.46	3.37	4.64	17.2	24.2	
n	3	2	3	2	3	3	

JEM-83GP-69

	ALA	VAL	LEU	ASP	PHE	GLU	location
X	.424	.150	.201	.501	.242	.148	N wall
S.D.	.011	.001	.001	.001	.021	.003	
C.V.%	2.59	6.71	.50	.20	8.67	2.03	
n	3	5	5	3	5	4	

JEM-83GP-70

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.422	.147	.216	.497	.242	.161	N wall
S.D.	.005	.005	.004	.007	.005	.007	
C.V.%	1.19	3.40	1.86	1.41	2.07	4.35	
n	3	3	3	2	3	3	

New Light Pit

80-171-1

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.569	.157	.204	.481	.244	.159	Unit 3
S.D.	.039	.028	.005	.024	.032	.015	
C.V.%.	6.83	17.9	2.42	5.0	13.1	9.43	
n	3	3	3	3	3	2	

80-171-2

Data disregarded for determination of D/L leucine chromatographic precision.

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.431	.174	.209	.521	.304	.175	Unit 3
S.D.	.039	.012	.139	.003	.031	.016	
C.V.%	9.07	6.94	66.5	0.57	10.2	9.14	
n	4	4	4	4	4	4	

80-171-2B

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.441	.149	.232	.515	.282	.156	Unit 3
S.D.	.019	0	.012	.004	.02	.003	
C.V.%	4.32	0	5.17	0.78	7.09	1.92	
n	3	1	3	3	3	3	

DFB-80-168

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.532	.230	.380	.556	.353	.168	Unit 2
S.D.	.01	.002	.002	.005	.054	.03	
C.V.%	1.88	0.87	0.53	0.90	15.3	17.9	
n	3	3	3	3	3	3	

Norris Bridge

JEM-83NB-56

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.728	.421	.557	635	.611	.304	75m
S.D.	.003	.011	.003	.098	.088		
C.V.%	0.41	2.62	1.97	0.47	16.0	29.0	
n	3	4	4	2	3	3	

JEM-83NB-25

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.705	.437	.520	.607	.437	--	75m
S.D.	.021	.093	.023	0	0	--	
C.V.%	2.98	21.3	4.43	0	0	--	
n	2	2	2	1	1	--	

JEM-83NB-10

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.767	.467	.564	.686	.504	.373	5m
S.D.	.076	.07	.036	.022	.030	.077	
C.V.%	9.44	15.0	6.38	3.21	5.92	20.6	
n	4	4	4	4	4	4	

80-165A

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.751	.399	.504	.655	.595	.381	?
S.D.	.037	.035	.006	.015	.027	.034	
C.V.%	4.93	8.77	1.19	2.28	4.54	8.92	
n	3	3	3	2	3	3	

all in ADMOS

JEM-83NB-126Z

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.723	.397	.513	.667	.551	.340	5m
S.D.	.018	.008	.012	.003	.002	.011	
C.V.%	2.48	2.01	2.34	0.45	0.38	3.24	
n	3	3	3	2	2	3	

JEM-83NB-12

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.762	.410	.512	.666	.552	.317	5m
S.D.	.054	.016	.004	0	.004	0	
C.V.%.	7.09	3.90	0.78	0	0.724	0	
n	2	2	2	1	2	2	

JEM-83NB-12M

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.737	.409	.537	.719	.574	.351	5m
S.D.	.002	.004	.008	0	.008	0	
C.V.%	0.27	0.98	1.49	0	1.39	0	
n	3	3	3	1	3	3	

Moyock Pit

JEM-83MP-131(2)

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.441	.194	.309	.537	.314	.195	spoil
S.D.	.007	.001	.002	.005	.003	.001	
C.V.%	1.59	0.52	0.65	0.93	0.96	5.64	
n	3	3	3	3	3	3	

JEM-83MP-97

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.569	.222	.350	.552	.355	.231	spoil
S.D.	.069	.048	.038	.056	.015	.046	
C.V.%	12.3	21.6	10.8	10.1	4.23	19.9	
n	4	4	4	4	3	4	

B.1 Sample Precision

Shown here are the data from samples JEM-83NB-126V through Z. Refer to Fig. 5-1 for placement of samples within the *Mercenaria* valve.

JEM-83NB-126V

	ALA	VAL	LEU	ASP	PHE	GLU
X	.695	.384	.489	.670	.521	.317
S.D.	.012	.016	.004	.001	.002	.026
C.V.%	1.73	4.17	0.82	0.15	0.38	8.20
n	3	3	3	2	2	2

JEM-83NB-126W

	ALA	VAL	LEU	ASP	PHE	GLU
X	.662	.346	.425	.684	.464	.317
S.D.	.013	.005	.005	.013	.006	.041
C.V.%	1.96	1.45	1.18	1.9	1.29	12.9
n	3	3	3	2	3	3

JEM-83NB-126X

	ALA	VAL	LEU	ASP	PHE	GLU
X	.665	.374	.540	---	.539	.320
S.D.	.014	.004	.046	---	.014	.007
C.V.%	2.11	1.07	7.89	---	2.60	2.19
n	2	2	2	---	2	2

JEM-83NB-126Y

	ALA	VAL	LEU	ASP	PHE	GLU
X	.291	.368	.452	.690	.487	.311
S.D.	.026	.008	.01	.008	.022	.003
C.V.%	3.76	2.17	2.21	1.16	4.52	0.96
n	3	3	3	3	3	3

JEM-83NB-126Z

	ALA	VAL	LEU	ASP	PHE	GLU
X	.723	.397	.513	.667	.551	.340
S.D.	.018	.008	.012	.003	.001	.002
C.V.%	2.48	2.01	2.34	0.45	0.38	3.24
n	3	3	3	3	2	2

APPENDIX C

STATISTICAL ANALYSIS OF FIELD DATA: *ANADARA*

Gomez Pit

JEM-83GP-86(1): hinge sample

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.425	.166	.289	.533	.353	.201	4'
S.D.	.019	.019	.01	.061	.025	.041	
C.V.%	4.47	11.5	3.46	11.4	7.08	20.4	
n	3	3	3	3	3	3	

JEM-83GP-86(2): whole shell sample

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.394	.158	.269	.466	.369	.155	4'
S.D.	.006	.004	.001	.009	.005	.007	
C.V.%	1.52	2.55	0.37	1.94	1.36	4.52	
n	4	4	4	4	4	4	

JEM-83GP-77: hinge sample

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.673	—	.238	.479	.324	.167	4'
S.D.	.187	—	.004	.004	.016	.025	
C.V.%	27.8	—	1.68	0.84	4.94	15.0	
n	3	0	3	3	3	3	

Norris Bridge

JEM-83NB-45(1): whole shell sample

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.625	.378	.536	.632	.654	.133	38m
S.D.	,09	.048	.019	.052	.654	0	
C.V.%	14.4	12.7	3.54	8.23	7.65	0	
n	3	3	3\	3	2	1	

JEM-83NB-45(2): hinge sample

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.649	.403	.519	.668	.636	.370	38m
S.D.	.013	.003	.007	.005	.01	.012	
C.V.	2.01	0.74	1.35	0.75	1.57	3.24	
n	4	4	4	4	4	4	

JEM-83NB-28: hinge sample

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.672	.383	.456	.661	=.586	.342	75m
S.D.	.014	.007	.004	.006	.006	.004	
C.V.%	2.08	1.83	0.88	0.91	1.02	1.17	
n	3	3	2	3	2	2	

JEM-83NB-15: whole shell sample

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.660	.372	.547	.631	.636	.408	5m
S.D.	.084	.048	.031	.014	.01	.01	
C.V.	12.7	12.9	5.67	2.22	1.57	8.10	
n	3	3	3	3	3	3	

Moyock Pit

JEM-83MP-102(1): whole shell sample

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.274	---	.152	.329	.251	.146	spoil
S.D.	.008	---	.004	.002	.015	.022	
C.V.	2.93	---	2.63	0.61	5.98	15.1	
n	3	---	3	3	3	3	

JEM-83MP-102(2): whole shell sample

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.312	.106	.191	.401	.256	.223	spoil
S.D.	.021	.004	.002	.007	.008	.005	
C.V.%	6.75	3.77	1.05	1.75	3.13	2.24	
n	2	2	2	2	2	2	

JEM-83MP-102(3): ?

	ALA	VAL	LEU	ASP	PHE	GLU	Location
X	.342	.112	.184	.443	.149	.214	spoil
S.D.	.034	.002	.007	.011	0	0	
C.V.%	9.94	1.79	3.80	2.48	0	0	
n	3	3	3	3	1	1	

APPENDIX D

AMINO ACID RATIOS

D.1 Amino Acid Ratios: Standards

SAMPLE	GLY/ALA	ASP/LEU
ILC-A1/24	1.296	0.815
	1.692	---
X	1.494±0.28	---
ILC-A3/29	0.891	6.257
	1.188	---
	1.258	---
	1.192	6.089
X	1.132±0.164	6.173±0.119
ILC-A4/11	1.252	7.115
	0.935	5.873
	1.108	7.969
	1.177	7.401
	1.451	10.561
	1.620	7.544
	1.646	7.501
	1.711	8.578
X	1.363±0.285	7.818±1.35
ILC-A4/30	1.122	8.541
	1.390	8.941
	1.529	7.940
X	1.347±0.207	8.479±0.504
ILC-A7/18 -		.378
	1.914	0.439
	1.849	0.445
	1.673	0.511
	1.019	0.532
X	1.614±0.409	0.461±0.062
ILC-A9/10	0.964	6.837
	0.959	6.822
X	0.962±0.004	6.829±0.012

SAMPLE	GLY/ALA	ASP/LEU
ILC-B1/18	0.931	5.129
	1.119	5.503
	1.077	4.656
	1.345	5.800
	1.548	---
	1.397	5.865
X	1.236±0.231	5.391±0.503
ILC-B2/10	0.961	4.364
	1.084	4.684
	0.677	3.488
	0.682	1.709
	0.927	4.381
	0.874	5.138
	0.682	3.959
X	0.839±0.165	4.336±0.571
ILB-B3/3	0.601	3.284
	0.546	1.941
	0.763	2.091
	1.054	4.496
X	0.741±0.228	2.953±1.19
ILC-B3/10	0.695	4.190
	0.672	3.613
	0.769	4.649
	0.742	4.416
	0.861	4.460
X	0.748±0.079	4.266±0.400

D.2 Amino Acid Ratios: *Mercenaria* Field Samples

Gomez Pit

SAMPLE	X GLY/ALA	X ASP/LEU
JEM-83GP-93	1.077±0.037	4.597±0.347
JEM-83GP-75	1.143±0.491	3.201±0.062
JEM-83GP-89M	0.951±0.140	2.660±1.362
JEM-83GP-69	1.356±0.034	6.716±1.020
JEM-83GP-70	1.259±0.199	5.943±1.427
JEM-83GP-95	2.088±0.328	9.399±0.387

New Light Pit

SAMPLE	X GLY/ALA	X ASP/LEU
80-171-1	2.127±0.955	13.923±7.735
80-171-2	1.767±0.145	7.837±1.544
80-171-2B	1.287±0.097	6.617±0.410
80-168	0.981±0.210	3.497±0.130

Norris Bridge

SAMPLE	X GLY/ALA	X ASP/LEU
JEM-83NB-56	0.724±0.047	2.164±0.815
JEM-83NB-25	0.647±0.386	0.766±0.855
JEM-83NB-10	0.301±0.091	2.763±1.172
80-165	0.832±0.053	7.473±0.293
JEM-83NB-126Z	0.957±	0.045
JEM-83NB-12	0.599±0.083	3.417 (n=1)
JEM-83NB-12M	0.531±0.029	2.635 (n=1)

Moyock Pit

SAMPLE	X GLY/ALA	X ASP/LEU
JEM-83MP-131(2)	0.967±0.122	2.454±0.085
JEM-83MP-97	0.871±0.108	3.580±0.174

D.3 Amino Acid Ratios: *Anadara* Field Samples

Gomez Pit

SAMPLE	X GLY/ALA	X ASP/LEU
JEM-83GP-86(1)	0.622±0.153	2.015±0.713
JEM-83GP-86(2)	1.004±0.157	2.330±0.126
JEM-83GP-77	1.957±0.799	5.763±2.44

Norris Bridge

SAMPLE	X GLY/ALA	X ASP/LEU
JEM-83NB-45(1)	0.750±0.022	2.353±0.609
JEM-83NB-45(2)	1.186±0.353	1.815±0.162
JEM-83NB-15	1.121±0.359	2.400±0.487

Moyock Pit

SAMPLE	X GLY/ALA	X ASP/LEU
JEM-83MP-102(1)	0.778±0.088	1.707±0.487
JEM-83MP-102(2)	1.597±0.055	3.170±0.178
JEM-83MP-102(3)	1.787±0.690	1.283±0.289

APPENDIX E

U-SERIES CORAL DATES USED FOR RADIOMETRIC CONTROL

Appendix 6 summarizes existing U-series coral dates for field sites of this study. All corals are g. *Astrangia.* All of these data have appeared previously in Cronin *et al.* (1981) and Mixon *et al.* (1982). There is a slight discrepancy in ages of the Norris Bridge and Gomez Pit sites between these two publications, due to differences in the reporting of U and Th concentrations for identical samples. As such, only dates from Cronin *et al.* (1981) will be used.

LOCALITY	U-SERIES DATE	COMMENTS
Norris Bridge	187 KA±20	Dates questionable on the basis of isotopic ratios
Gomez Pit	75 KA±5	Collected nr Norfolk Fm./ "Kempsville" Fm. contact
Moyock Pit	72 KA±4	Collected from spoil pile
New Light Pit	74 KA±4	Collected nr Norfolk Fm./ "Kempsville" Fm. contact

Table E-1: U-Series Coral Dates from the Southeastern Virginia/North Carolina Coastal Plain. U-Series dates originally appeared in Cronin *et al.* (1981) and later in Mixon *et al.* (1982)

all listed under 06029

APPENDIX F

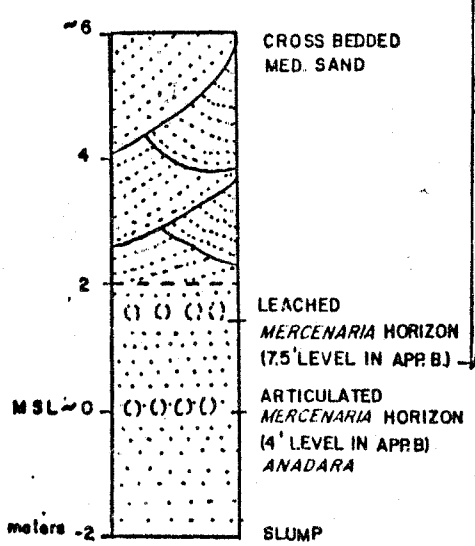
FIELD LOCATIONS

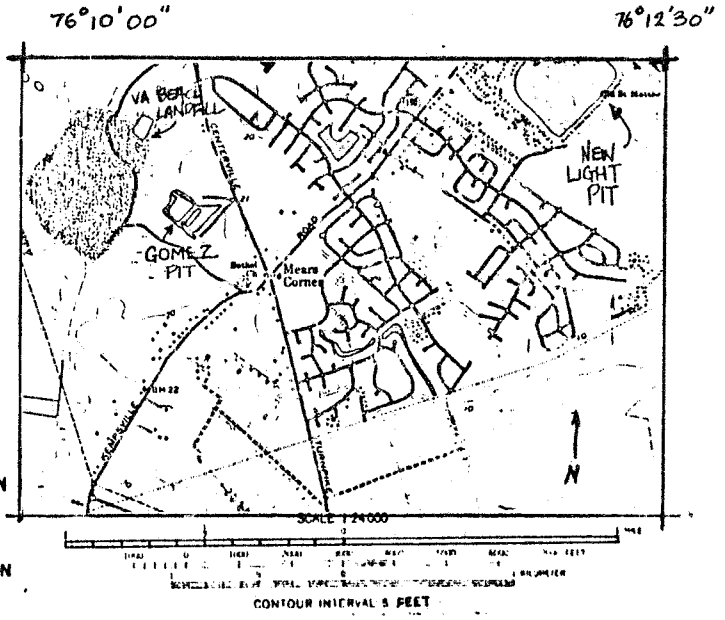
F.1 Gomez Pit

Gomez Pit, Mears, VA
35°47'15" N latitude
76°10'20" W longitude
Kempsville 7.5 minute quad
1965, photorevised 1980

All samples from this exposure are used to calculate the "upper strata" mean D/L leucine values for *Mercenaria* and *Anadara* found in Table 5-2 (this thesis).

SAMPLE	D/L LEUCINE
JEM-83GP-89M	0.231
JEM-83GP-89	0.415
JEM-83GP-93	0.217
JEM-83GP-95	0.210
JEM-83GP-75	0.203
JEM-83GP-69	0.201
JEM-83GP-70	0.216
Anadara	
JEM-83GP-86(2)	0.269
JEM-83GP-77	0.238





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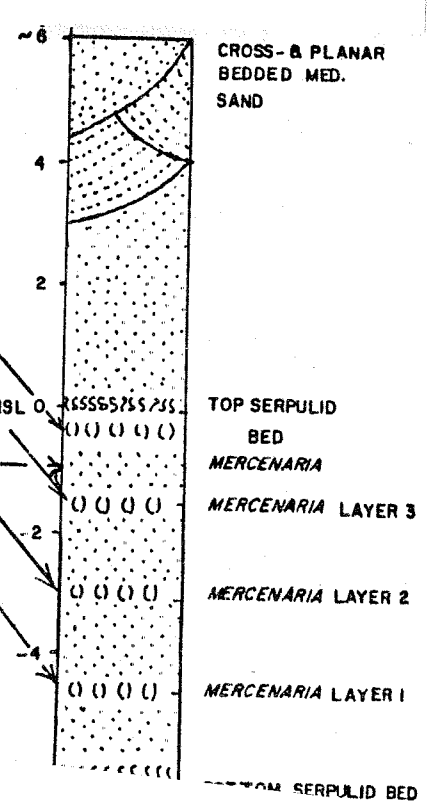
06030

F.2 Stratigraphic Section, Southwest Wall, Gomez Pit

Data shown here were not analyzed in time for inclusion in the statistical analysis section of this study.

	SAMPLE	X D/L LEUCINE
	Mercenaria	
Upper strata	JEM-83GP-138	0.237
	JEM-83GP-141	Sample lost
	JEM-83GP-137	0.281
	JEM-83GP-140	0.251
	Approximate location of 75 ±5 KA U-series coral date coral date of Cronin *et al.*, 1981.	
Lower strata	JEM-83GP-136	0.520
	JEM-83GP-135	0.538
	JEM-83GP-139	0.531



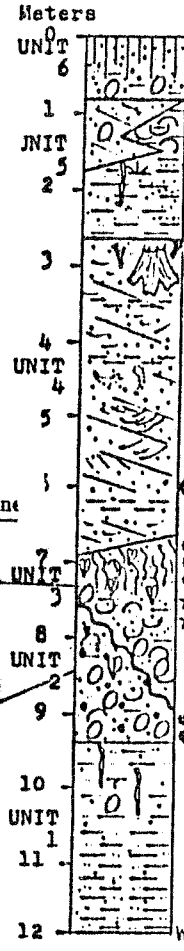
All recorded in MANOB as 84GP ---

12/1/12

F.3 New Light Pit

from Belknap (1979)

NL - Williams Paving Company Pit, New Light, Va.
SW Wall, less active part of excavation.
C 1/9 Kempsville, Va. 7½' Quad.
36°47.5'N, 76°11.2'W, Elev. 6 m.
Described by: D.F. Belknap, 8/5/76; 3/26/77; 4/26/77; and 8/6/77



Modern soil zone in forested area
Gravelly sandy mud, deep brown, very few scattered shell frags., tree roots

M sand, gravelly, orange mottled white; interfingers laterally with interlayered mud and sand laminae, locally abundant oysters, burrowed. Plant fragments common. Fossils appear abraded and broken, poorly preserved, reworked ?

Sharp contact, overlying tree stumps and roots in lower unit
(Backshore to dune ?)

F sand, well sorted, clean white. Trough and planar cross beds, ripples abundant near top of section, abundant heavy minerals. Shell ghosts.

Several mud layers up to 10 cm thick contain plant debris. Suggest runnels or tidal flat.

A few ophiomorpha (?) burrows.

MSL
(Foreshore ?)

Serpulid zone: F-M sand, mod. sorted, silty. Gravel and cobbles at base. Abundant articulated Mercenaria, Tagelus in growth position, Busycon, Crassostrea, arks, serpulid worms, coral, bones, and many other mollusks. (Bay-Lagoon ?)

Sharp erosional contact over:
C-VC gravelly sand, gray-green, orange mottl. Shells common, Mercenaria bed near contact, common Spisula. (Littoral zone ?)

Contact covered
Gray M-F sandy pebbly mud, burrowed, at least 2 m thick to water level in drainage ditch. Unit becomes fossiliferous on N wall of pit, as gravelly sand facies pinches out; collected 1/30/78.

Water level 8/6/77

UNIT 1 - Great Bridge ?; UNIT 2 - ? ; UNIT 3, Norfolk; UNIT 4 - Kempsville ; UNIT 5, Sandbridge; UNIT 6, ?
Correlations from sections of Oaks and Coch, 1973, WP.
See Appendix B, p. for sample numbers in this section

Figure A49b.

Applecrates

	SAMPLE	D/L leucine
unit 3	JW-80-171-1	0.204
	JW-80-171-2	0.209
	JW-80-171-2B	0.232
unit 2	DFB-168	0.380

Published *Mercenaria* analyses

(Wehmiller and Belknap, Table 2, 1982;

Unit 2 data from Belknap, p.526, 1979).

	SAMPLE	D/L leucine
unit 3	JW-80-171-1	0.27
	JW-80-171-2	0.29
unit 2	DFB-168	0.422

06024

06025

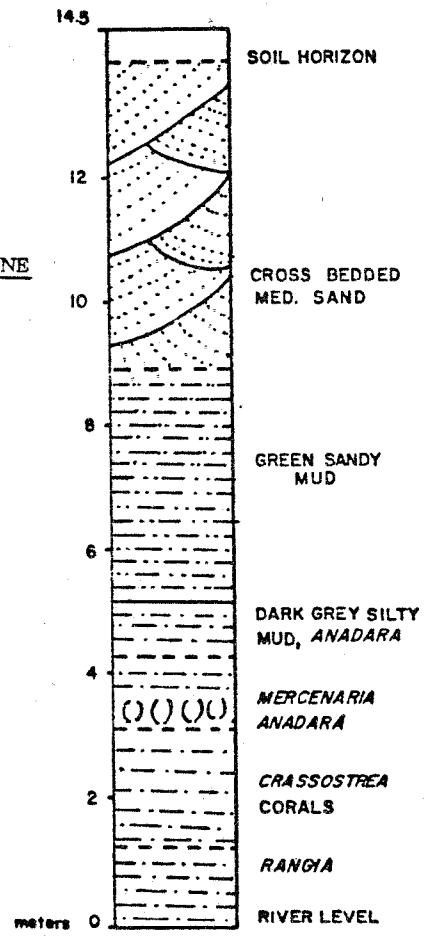
06 RR

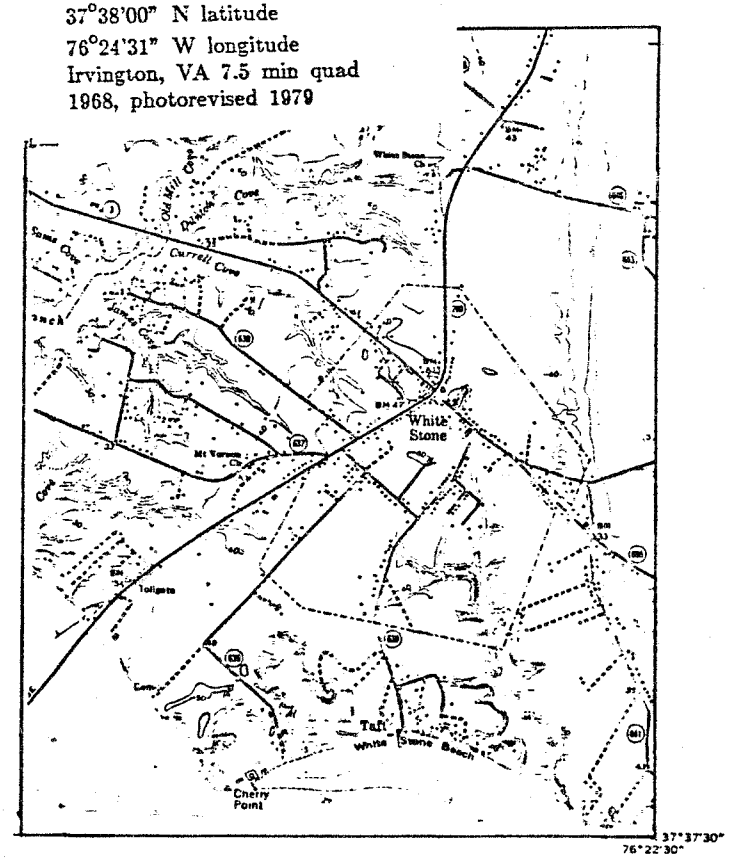
F.4 Norris Bridge

Norris Bridge, north bank of the Rappahannock River east of Norris Bridge. Composite section of profiles from 75m, 38m, and 5m from the reference point.

37°38'00" N latitude
76°24'31" W longitude
Irvington, VA 7.5 min quad
1968, photorevised 1979

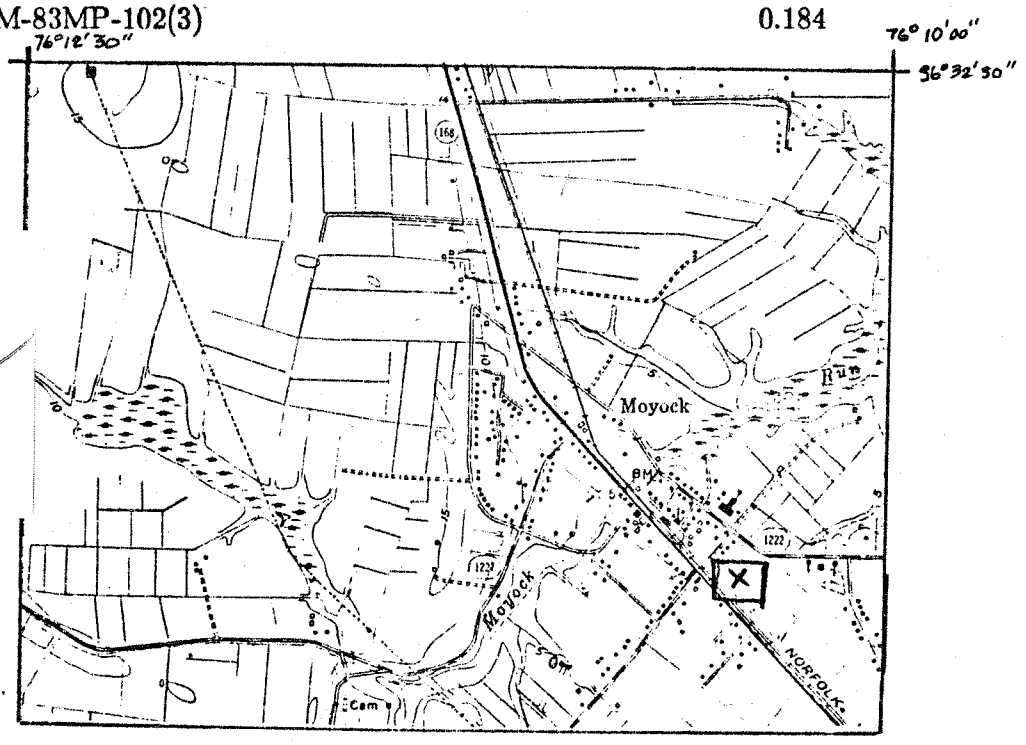
SAMPLE	D/L LEUCINE
Mercenaria	
JEM-83GP-56	0.557
JEM-83NB-25	0.520
JEM-83NB-10	0.564
JEM-83NB-126Z	0.513
JEM-83NB-12	0.512
JEM-83NB-12M	0.537
80-165A	0.504
Anadara	
JEM-83NB-45(1)	0.536
JEM-83NB-45(2)	0.519
JEM-83NB-28	0.456
JEM-83NB-15	0.547





Moyock Pit, Moyock, N.C.
Spoil piles adjacent to pig farm,
Rte 168.
36°47'15" N latitude
76°10'20" W longitude
Moyock, VA-NC 7 5 min quad
1954, photorevised 1971

SAMPLE	D/L LEUCINE
Mercenaria	
JEM-83MP-131(2)	0.309
JEM-83MP-97	0.350
Anadara	
JEM-83MP-102(1)	0.152
JEM-83MP-102(2)	0.191
JEM-83MP-102(3)	0.184



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APPENDIX G

EFFECTIVE QUATERNARY TEMPERATURE (EQT) CALCULATIONS

As stated in Chapter 7, EQTs are calculated for use in the non-linear kinetic model (Fig. 7-1). The following assumptions are made in these calculations:

1. $-\Delta T_{fg} = 8^{\circ}$ to $12^{\circ}C$ (Wehmiller and Belknap, 1979). $-\Delta T_{fg}$ is the full-glacial temperature reduction for $33^{\circ}N$ latitude.

2. Norfolk, VA mean annual air temperature = $16^{\circ}C$ (p. 498; Belknap, 1979).

3. $\log k = 15.77 - 5939/T^{\circ}K$ (Bada and Schroeder, 1972). This modified Arrhenius equation defines the temperature dependence of the isoleucine racemization reaction, using an activation energy of 27 to 30 kcal/mole.

4. Use of the Square-Wave Temperature History model (Fig. G-1).

For shells of 75 KA age:

$-\Delta T_{fg} = 8^{o}$

Air Temp (^{o}C)	Duration	k	Weighted Avg. Factor
16 ^{o}C	10 KA	1.7×10^{-5}	0.133
8	60	0.4426×10^{-5}	0.8
13	5	0.7397×10^{-5}	0.066

$$1.7 \times 10^{-5} (0.133) = 2.261 \times 10^{-6}$$
$$0.4426 \times 10^{-5} (0.8) = 3.541 \times 10^{-6}$$
$$0.7397 \times 10^{-5} (0.066) = 4.882 \times 10^{-7}$$

6.2902×10^{-6} weighted average rate constant (k)

using 3.), above:

$$\log (6.2902 \times 10^{-6}) = 15.77 - 5939/T^{o}K$$

$T^{o}K = 283.19 \quad T^{o}C = 10 = EQT$

$-\Delta T_{fg} = 12^{o}$

Air Temp (^{o}C)	Duration	k	Weighted Average Factor
16 ^{o}C	10 KA	1.7×10^{-5}	0.133
4	60	0.21937×10^{-5}	0.8
12	5	0.6241×10^{-5}	0.066

$$1.7 \times 10^{-5} (0.133) = 2.261 \times 10^{-6}$$
$$0.21937 \times 10^{-5} (0.8) = 1.755 \times 10^{-6}$$
$$0.6241 \times 10^{-5} (0.066) = 4.119 \times 10^{-7}$$

4.428×10^{-6} weighted average rate constant (k)

using 3.), above:

$$\log (4.428 \times 10^{-6}) = 15.77 - 5939/T^{o}K$$

$T^{o}K = 281.15 \quad T^{o}C = 8^{o} = EQT$

For shells of 125 KA age:

$-\Delta T_{fg} = 8^{o}$

Air Temp (^{o}C)	Duration	k	Weighted Average Factor
16°C	10 KA	1.7×10^{-5}	0.08
8	60	0.4426×10^{-5}	0.48
13	45	1.0355×10^{-5}	0.36
16	10	1.7×10^{-5}	0.08

$$1.7 \times 10^{-5}(0.16) = 2.720 \times 10^{-6}$$
$$0.4426 \times 10^{-5}(0.48) = 2.124 \times 10^{-6}$$
$$1.0355 \times 10^{-5}(0.36) = 3.728 \times 10^{-6}$$
$$8.572 \times 10^{-6}$$ weighted average rate constant (k)

using 3.) above:

$$\log (8.572 \times 10^{-6}) = 15.77 - 5939/T^{o}K$$

$T^{o}K = 284.98 \quad T^{o}C = 13.8 = EQT$

$-\Delta T_{fg} = 12^{o}$

Air Temp (^{o}C)	Duration	k	Weighted Average Factor
16°C	10 KA	1.7×10^{-5}	0.08
4	60	0.21937×10^{-5}	0.48
12	45	0.6241×10^{-5}	0.36
16	10	1.7×10^{-5}	0.08

$$1.7 \times 10^{-5}(0.16) = 2.720 \times 10^{-6}$$
$$0.21937 \times 10^{-5}(0.48) = 1.053 \times 10^{-6}$$
$$0.6241 \times 10^{-5}(0.36) = 2.247 \times 10^{-6}$$
$$6.020 \times 10^{-6}$$ weighted average rate constant (k)

using 3.), above:

$$\log (6.020 \times 10^{-6}) = 15.77 - 5939/T^{o}K$$

$T^{o}K = 282.94 \quad T^{o}C = 9.79 = EQT$

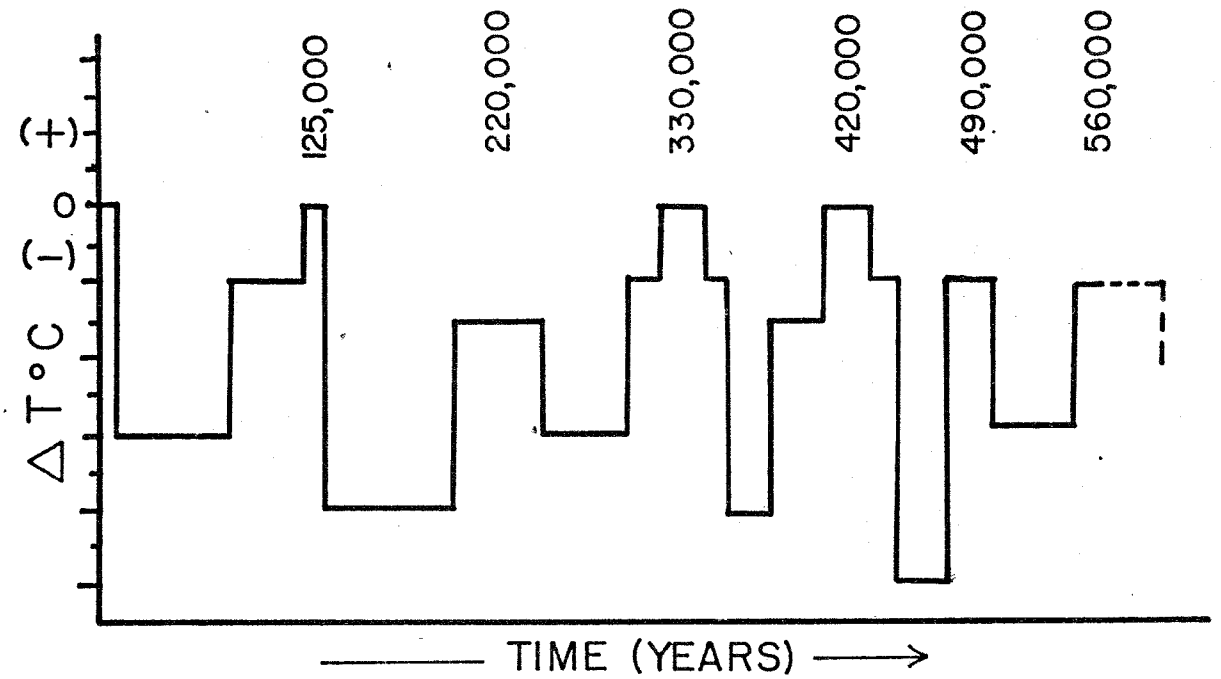

Figure G-1: Square-Wave Temperature History Model.
Redrawn from p. 495, Belknap (1979).