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APPLICATION OF AN ACETATE PEEL TECHNIQUE TO ANALYSIS OF GROWTH PROCESS IN BIVALVE UNIONID SHELLS

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INTRODUCTION

Since many southeastern prehistoric sites were evidently occupied on a seasonal basis, archeological research has been increasingly concerned with the patterning as well as the nature and extent of occupation of aboriginal settlement systems. At the present time, few techniques of any kind are available for determining the season of occupation and resource utilization of prehistoric sites. Traditional archeological analysis has relied on a range of techniques from forthright speculation based on the presence or absence of certain animal or plant species at sites to a more empirical study of growth increments on fish scales. Many archeological sites in the southeastern United States characterically contain large quantities of shells which were utilized and discarded by prehistoric peoples. The unionid bivalve shells in these deposits presumably reflect the annual and seasonal growth increments of the mollusks which produce them. If a technique could be devised which would furnish a reliable estimate of the season at which the shells of these ancient animals were harvested, it would also be a most useful archeological tool. This paper is a progress report on an attempt to determine the season of death of unionids by applying an acetate peel technique to the analysis of the growth process in the shells. Since this study is primarily attempting to test the usefulness of the technique with respect to freshwater unionid shells, only modern-day shells have been examined to date.

GROWTH AND FORMATION OF THE SHELL

Some of the most intensive shell growth studies on unionid mussels took place early in this century as a result of the button industry (Lefevre and Curtis, 1912; Isely, 1914; Coker, *et al.* 1921; Chamberlain, 1930). These studies found that if the margin of the mantle, where shell growth occurs, withdraws within the shell to such an extent that its former continuity with the distal edge of the shell is severed, then, when growth resumes, there is apt to be an overlapping or doubling up of the outer two shell layers. This overlapping, and the resultant change in rate of calcium carbonate deposition, is often manifested by dark bands. These may be formed annually (during the winter season) or as the result of more singular disturbances such as extreme water temperature fluctuation and/or temporary stranding of the animal. Interruption increments corresponding to the winter season differ from those corresponding to unfavorable conditions in that the latter appear to have only single duplications of the outer shell layers while the former has several. Chamberlain (1930) argues that these duplications can be distinguished by careful microscopic examinations of cross sections of the prismatic layers of the shells. With the acetate peel technique, I am testing Chamberlain's conclusions.

PREPARATION AND ANALYSIS OF SAMPLES

The technique is based on work done in New Zealand with marine mollusks by Coutts (1970) and the procedure is summarized by Kummel and Raup (1965). Recent bivalves were collected throughout the year from various locations in Arkansas. These locations (the Upper Buffalo River, the Upper and Lower White River, the Lower St. Francis River, and several smaller permanent streams) correspond with the locations of numerous archeological sites, many of which show evidence of extensive molluscan exploitation during prehistoric times.

Shell growth was studied by means of acetate peels. After embedding the shells in dental stone (a dense plaster), I cut them at right angles to the growth increments (along the umbonal axis), then ground and polished them to obtain smooth, flat surfaces. These surfaces were etched with 5% hydrochloric acid for 15 seconds to one minute. In some cases, Alizarine Red-S was used to stain the surface producing negative surface impressions of the cross sections. These peels were examined at magnifications ranging from 10 to 200 power.

The determination of the season of death of a unionid is accomplished by measuring the distance between the annual growth recession rings. Chamberlain (1930) found that in many species, the distance between rings decreases logarithmically with respect to age. Therefore, it should be possible to extrapolate the distance from the last ring to the next one which would have been formed if the organism had lived. By comparing this calculated distance to the actual edge of the shell, the time of year when death occurred could be estimated.

RESULTS AND CONCLUSIONS

To date, acetate peels of approximately 75 unionids representing 13 species have been made. No list of species is provided at this time due to the rather generalized results presented here; however, a complete list of verified identifications will be published later. The peels have possibly revealed the presence of two kinds of growth arrest markers: (1) the annual interruption ring with its several duplications of the outer shell layers; and (2) the intra-annual interruption ring with its single duplication (Plates I, II, and III). Difficulty, however, has occurred in attempting to distinguish between these two markers in all of the specimens analyzed. For instance, it has become apparent that some unionid bivalve shells are better suited for this type of study than are others. Although bivalves of the genus *Quadrula* have thick shells and are often preserved in archeological deposits, their relatively long life spans and slow shell growth beyond the juvenile stage result in the production of growth lines so close together that differentiation by means of this technique is often difficult. The evidence to date suggests that the faster growing species of the genus *Lampsilis* might prove promising; however, further study will be necessary to determine the more suitable species for shell growth analysis. It has been also observed that the growth interruption increments tend to be narrower and less distinct toward the hinge of the valve (i.e., those formed during juvenile growth), resulting in greater difficulty in distinguishing the two types of rings.

ACKNOWLEDGEMENTS

Identifications were verified by Dr. Louise Kraemer of the University of Arkansas zoology department. Voucher specimens available at the University of Arkansas Museum, Fayetteville.

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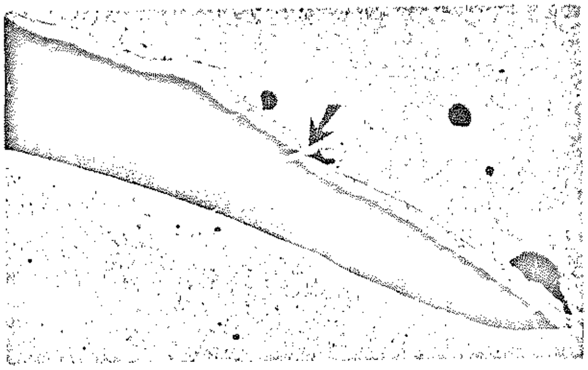

Plate I-A. An acetate peel of the cross section of the shell of a *Quadrula pustulosa* (growth increment indicated by arrow).

Plate I-B. An enlargement of an acetate peel of the cross section of the shell of a *Quadrula pustulosa* (not the same specimen as above) showing a prominent annual growth recession increment.

Plate II-A. An acetate peel of the cross section of the shell of a *Uniomerus tetralasmus* near the distal margin (enlargement section shown below).

Plate II-B. An enlargement of an acetate peel of the cross section of the shell of a *Uniomerus tetralasmus* (same specimen as above) showing growth recession increments as manifested in the prismatic and nacreous layers.

Plate III-A. An acetate peel of the cross section of the shell of an *Arcidens confragosus* showing growth recession increments in the prismatic layer.

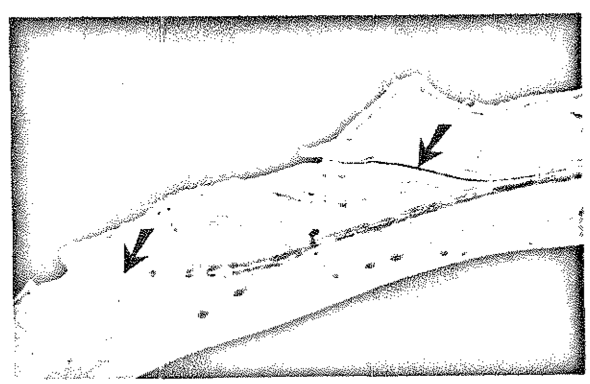

Plate III-B. An acetate peel of the cross section of the shell of a *Quadrula quadrula* showing several prominent growth recession increments.

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VILLOSA LIENOSA (CONRAD, 1834) IN OHIO

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Villosa lienosa (Conrad, 1834), a naiad species common in Gulf coastal states, has been collected in several midwestern states but has never been reported as occurring in Ohio. Recently, we and other collectors at The Ohio State University Museum of Zoology (OSUM) have found populations of this species in five separated streams in the southern third of Ohio (Fig. 1). These streams are East Fork of the Little Miami River, Whiteoak Creek, Scioto Brush Creek, Little Salt Creek and one tributary of Symmes Creek. These five streams are all relatively small (2-5m wide) and of low gradient (less than 12 ft./mi.) (Krolczyk, 1960) with sand and/or mud bottoms where the *V. lienosa* specimens were collected. These habitat observations are consistent with previously recorded habitat descriptions for this naiad (Clench and Turner, 1956; Parmalee, 1967).

Literature records of northern populations of *V. lienosa* exist for Illinois (Baker, 1922), Indiana (Blatchley and Daniels, 1903; Goodrich and van der Schalie, 1944) and Kentucky (Wilson and Clark, 1914; Ortmann, 1926; Stansbery, 1965) but not for Pennsylvania. The OSUM has one lot from the Hughes River in West Virginia, which appears to be a new record for that state. All of these sites are plotted on Fig. 1.

We have compared the known distribution records of *V. lienosa* with several stream-related factors in an attempt to explain why this naiad does not occur throughout the upper Ohio River system. Blatchley and Daniels (1903) mention that *V. lienosa* was common in the canal and White River at Indianapolis. Canals fit our concept of the habitat of *V. lienosa* and, approximately 125 years ago, canals connected the major midwestern drainage systems, affording potential access to more northern streams in several states. Our comparison of the canal systems and the distribution records for *V. lienosa* failed to reveal any clear relationships.

A similar comparison was made of the distribution records for *V. lienosa* with bedrock composition. Midwestern bedrocks vary widely in their pH characteristics and are deposits of most Paleozoic periods. We found that the range of this naiad species extends over all bedrock types present in this area.

Geologic evidence suggests that, prior to the Pleistocene, midwestern stream drainage patterns were considerably different from those of the present. We compared the distribution of *V. lienosa* with the Teays drainage, the most widely accepted concept of a pre-glacial drainage pattern. The correlation we found was best for parts of streams south of the glacial boundary— —areas where drainage patterns have changed very little.

Our final attempt was to correlate the distribution pattern of *V. lienosa* with the glacial patterns of the midwest. Three of the sites in Ohio are located south of any glacial boundary; the other two sites are located south of the Wisconsin glacial boundary, but within the area once covered by the Illinoian glacier. In Indiana, four sites are south of the Wisconsin glacial boundary (with one site in unglaciated terrain), while the other four are north of the Wisconsin boundary. In the state of Illinois the only records of which we are aware are those from the Big Vermilion River (Baker, 1922), a small stream located in a Wisconsin-glaciated area.

Goodrich and van der Schalie (1944) state that southern Indiana marks the northern limit of the range of *V. lienosa*. We have extended the known range of this species east into Ohio and West Virginia, and slightly to the north in Illinois, but we have not changed their basic observation that this area constitutes the northern limit of the range. We

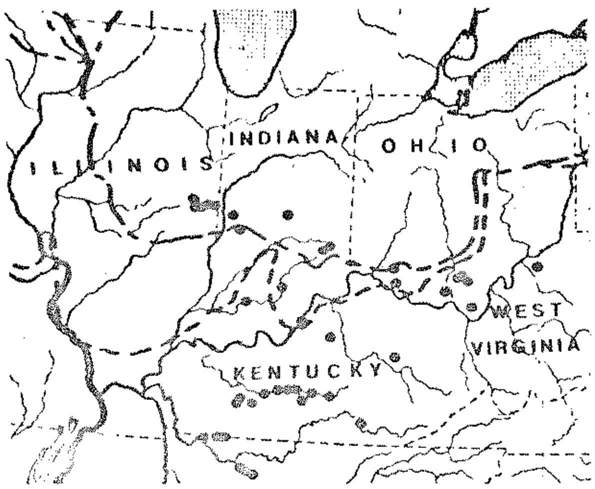

Figure 1. Localities at the northern edge of the range of *Villosa lienosa* (Conrad, 1834) taken from published accounts (see text) and from specimens at the Ohio State University Museum of Zoology. The long- and short-dashed line indicates the Wisconsin glacial boundary; the short-dashed line indicates the Illinoian glacial boundary.

have not examined specimens housed in other museums, nor have we collected widely in small streams outside of Ohio. Additional records from either of these sources would provide a better concept of the complete distribution pattern. Known records indicate that the distribution pattern of *V. lienosa* has at least some relationship with the Wisconsin glacial boundary: every known collection has been taken south of, or only slightly north of, this glacial feature. We are unaware of any characteristics of Wisconsin-glaciated areas which would preclude the introduction of *V. lienosa* by some fish host, or which would prevent the survival of *V. lienosa* specimens once they were introduced. Further study and collection of additional specimens will be necessary before we can determine more precisely what factor or factors form this apparent barrier.

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