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A TECHNIQUE TO SEPARATE THE ANNUAL LAYERS OF A NAIAD SHELL (MOLLUSCA, BIVALVIA, UNIONACEA) FOR ANALYSIS BY NEUTRON ACTIVATION[1]

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Trace metals discharged into our streams and rivers, even though usually in small concentrations, are often toxic to some of the aquatic life. Because the concentrations are so minute and vary greatly with time, detection of the source is not feasible by ordinary chemical monitoring. In such situations analysis of an aquatic organism that would concentrate the metals to a measurable level and integrate the variations in concentration over its growing season can indicate the presence of toxic metals. If the indicator organism is relatively immobile, analysis of specimens from different sites can help locate the effluent responsible.

Naiades are relatively immobile, are widely distributed (Simpson 1914) and are known to concentrate many trace metals in their shells (Girardi and Merlini 1963). But what makes the naiad an especially good indicator organism is the fact that the shell is deposited in annual layers (Chamberlain 1930). If these layers could be isolated and analyzed individually, annually integrated trace metal concentrations could be determined for a specific location as far back in time as the age of the naiad would permit.[2] This study was undertaken to develop an accurate shell separation technique and an appropriate analytical technique to make the historical information in the naiad shell available.

Activation analysis was chosen as our analytical technique because of its sensitivity to metals and because it requires only a small amount of sample material. For activation analysis of naiad shells, at least 0.5 gram of sample material is required (Merritt 1974).

Before developing a method for separating the naiad shell into annual layers, an understanding of shell formation was necessary. A naiad shell actually has four layers formed by different areas of the mantle and each layer has its own annual layers. Two thin outer layers are formed by the free ventral edge of the mantle: the outer protein membrane, the periostracum, and under the periostracum, the crystalline prismatic layer (Wilbur 1964). The layer forming the bulk of the shell, the peripheral nacre, is deposited by the surface of the mantle ventral, or peripheral, to the pallial line. The thin laminar nacre is deposited by the surface of the mantle dorsal, or central, to the pallial line (Nelson 1964). (See Fig. 1.)

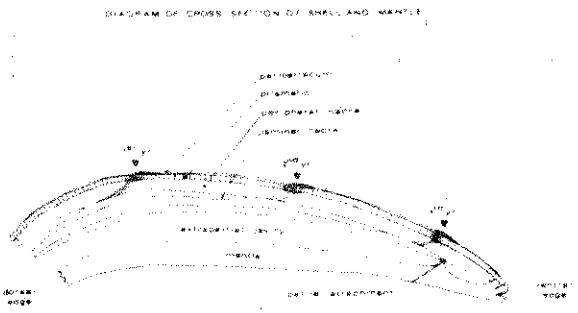

Fig. 1. A diagram of the shell and mantle showing the four shell layers with their annual layers in cross section.

The mantle first deposits the periostracum which serves as an organic matrix on which $CaCO_3$ is crystallized into the three crystalline shell layers (Bevelander and Benzer 1948, Taylor et al., 1969).

As in trees, the annual layers in naiades are a result of seasonal changes in growth rate. During periods of low temperature, the mantle withdraws and ceases shell formation (Coker et al. 1921). Repeated interruptions in shell formation during the spring and fall result in an area of discontinuity visible as a dark line. The greater amount of periostracum at this border results in an area higher in protein than the other parts of the shell. Theoretically, if the shell is baked

[2] NOTE: Some naiades commonly live 15-25 years and some have been estimated at 40-60 years (Isely 1931, Stansbery 1961).

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at high temperatures, the protein of the periostracum will ash and not the $CaCO_3$, leaving a weak spot or crack that delineates the part of the shell formed in a single growing season.

Utilizing these facts, Nelson (1964) described a baking method for separating annual layers. He cut each valve in half medially and ashed it in a muffle furnace at 400°C for four hours. The annual rings of each layer were then separated with a scalpel. A second separation technique was described by Pahl (1967). He also cut each valve in half medially but ashed it in a muffle furnace at 500°C for 10 minutes and separated only the nacre ir to the annual layers using a sharp probe and forceps.

Although both Nelson and Pahl sawed the shells in half, we attempted to separate the layers on a whole baked valve. The layers, however, stuck together at the edges just anterior and posterior to the umbo. When pressure was applied to separate the layers, the whole shell crumbled. Sawing the shell in half removes one edge enabling better separation. We found that if the valves are cut from the umbo to the ventral edge at both the anterior and posterior margins, the wedge produced separates far better than either the whole valve or a half. (See Fig. 2.) The shells were cut with an automatic saw using a carborundum-charged blade which produced a smoother cut and was a great deal easier than a hack saw.

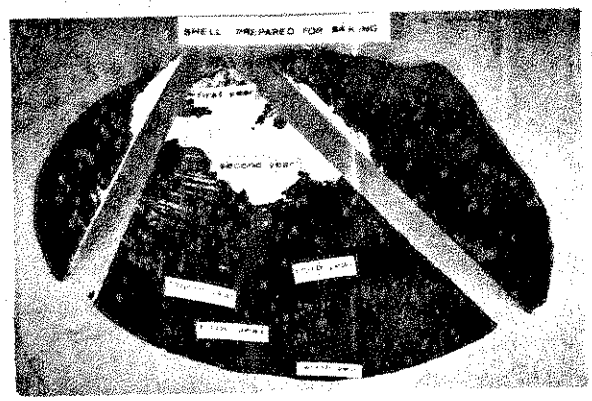

Fig. 2. One valve of a *Lasmigona complanata* (Barnes 1923) sawed in preparation for baking.

The shells baked by the shorter method separated much more easily than those baked by the long method. Those wedges baked longer developed many separations within an annual layer and tended to crumble. The prismatic layer was much easier to separate from the peripheral nacre in the wedges baked for 10 minutes. We found that shells separated well even when baked for five minutes at 600°C. (See Fig. 3.)

Because the prismatic layer is much too thin and does not develop cracks at the annual layers, it was not used for analysis. The laminar nacre (formed within the pallial line) is too thin in some species and tends to flake apart easily. On the other hand, the layers of the peripheral nacre usually weighed 0.5 gram except for the first year of growth and could be separated easily with forceps and a sharp probe.

Samples of peripheral nacre were prepared from five species collected in the Muskingum River in Ohio: *Lasmigona complanata* (Barnes, 1823), *Quadrula quadrula* (Rafinesque, 1820), *Amblema plicata plicata* (Say, 1817), *Obliquaria reflexa* (Raf., 1820), and *Potamilus alatus* (Say, 1817). Of these, *P. alatus* and *L. complanata* were the easiest to separate.

By cutting the valves into wedges and baking them for five minutes at 600°C, the peripheral nacre can be separated into annual layers. An analysis can be made of each layer, except the first, producing an historical record of metal concentrations which were present in that naiad's environment. Analysis of naiad shells collected before and after the installation of a suspect pollutant could determine the resultant change in water quality. Using recently collected naiades, the difference between metal concentrations above and below a suspect effluent could be determined. Analysis of a recently deposited layer could indicate the presence of hazardous elements before they reach a damaging concentration, and indicate the source and the range downstream. The full potential of information available from naiad shells remains to be determined.

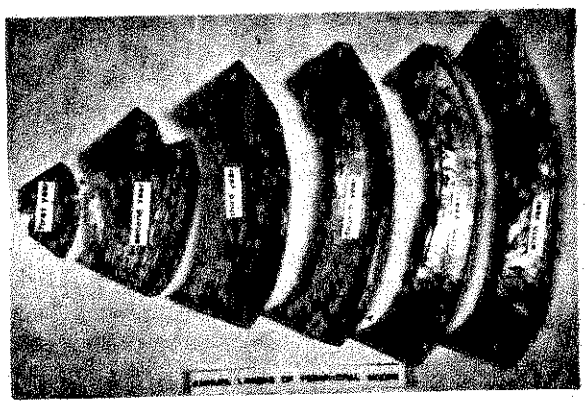

Fig. 3. The peripheral nacre from the center wedge shown in Fig. 2. The wedge was baked in a muffle furnace at 600°C for five minutes and the layers separated with a needle and forceps.

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