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Appendix 1

Preparation and Examination of Skeletal Materials for Growth Studies

Part A: Molluscs

1. Preparation of Acetate Peels and Fractured Sections for Observation of Growth Patterns within the Bivalve Shell

MICHAEL J. KENNISH, RICHARD A. LUTZ, and DONALD C. RHOADS

1. Acetate Peels

Throughout various chapters of this book (especially Chapters 6 and 7), reference has been made to acetate-peel replicas of polished and etched bivalve shell sections. Such peel replicas are easily and rapidly prepared; seven procedural steps are involved: (1) embedding, (2) sectioning, (3) grinding, (4) polishing, (5) acid-etching, (6) washing and drying, and (7) application of acetone and acetate. We will describe these steps and give examples of specific products various workers have used.

1.1. Embedding

As an initial step, individual shell valves are embedded in an epoxy resin to prevent fracturing during sectioning. Rhoads and Pannella (1970) have reported success using Epon 815 resin and DTA curing agent from

MICHAEL J. KENNISH • Environmental Affairs Department, Jersey Central Power & Light Company, Morristown, New Jersey 07960. RICHARD A. LUTZ • Department of Oyster Culture, New Jersey Agricultural Experiment Station, Cook College, Rutgers University, New Brunswick, New Jersey 08903. DONALD C. RHOADS • Department of Geology and Geophysics, Yale University, New Haven, Connecticut 06520.

the Miller Stephenson Chemical Co., Inc., Danbury, Connecticut 06810. A modification of their method follows.

The epoxy solution should be prepared by thoroughly mixing the reagents in a ratio of 1 part DTA curing agent to 10 parts Epon 815. A greater proportion of curing agent should be avoided due to the exothermic reaction of the curing agent–resin mixture. Each pouring of epoxy should be restricted to thicknesses no greater than 2 cm; pouring thicknesses greater than this may result in a violent exothermic reaction. Embedding of large shells, which require greater thicknesses of epoxy, should be accomplished by building up 2-cm-thick layers one at a time. Disposable plastic containers (e.g., PEEL-A-WAY tissue embedding molds, Lipshaw Manufacturing Co., 7446 Central Avenue, Detroit, Michigan 48210) can be used as nonsticking molds for the epoxy. After a shell valve is placed in the liquid epoxy mixture, the entire container should be placed under vacuum. After approximately 5 min, the vacuum is released, and the evacuation is then repeated for another 5 min. This procedure should be repeated until no air bubbles remain in the epoxy. The mixture may then be left at ambient pressure to cure. Curing time for the mixture is approximately 6–8 hr at room temperature.

1.2. Sectioning

After the epoxy has hardened, the embedded shell may be sectioned along the desired axis, using a diamond rock saw (e.g., various models of saws from Buehler Ltd., 2120 Greenwood Street, P.O. Box 830, Evanston, Illinois 60204; solid rim blades with No. 1 concentration of diamonds from Felker Operations, Dresser Industries, Inc., 1900 South Crenshaw Boulevard, Torrance, California 90509). Because the epoxy is transparent, it is possible to examine the embedded shell and to define a transect for this section; this transect may be scratched directly on the epoxy surface to serve as a guide during sectioning. In the majority of studies of internal bivalve growth patterns, the valve or valves are sectioned along the axis of maximum growth (Fig. 1, a–b) (also see Chapter 1). This cut is oriented so that growth increments intersect the plane of section at right angles (Fig. 1).

1.3. Grinding

Once cut, valve cross sections are ground sequentially on cast iron lapidary wheels using carborundum powders in the following order of grit sizes: 120, 240, 320, 400, and 600 (e.g., Buehler carborundum grits). If the saw cut is sufficiently smooth, it may not be necessary to use the

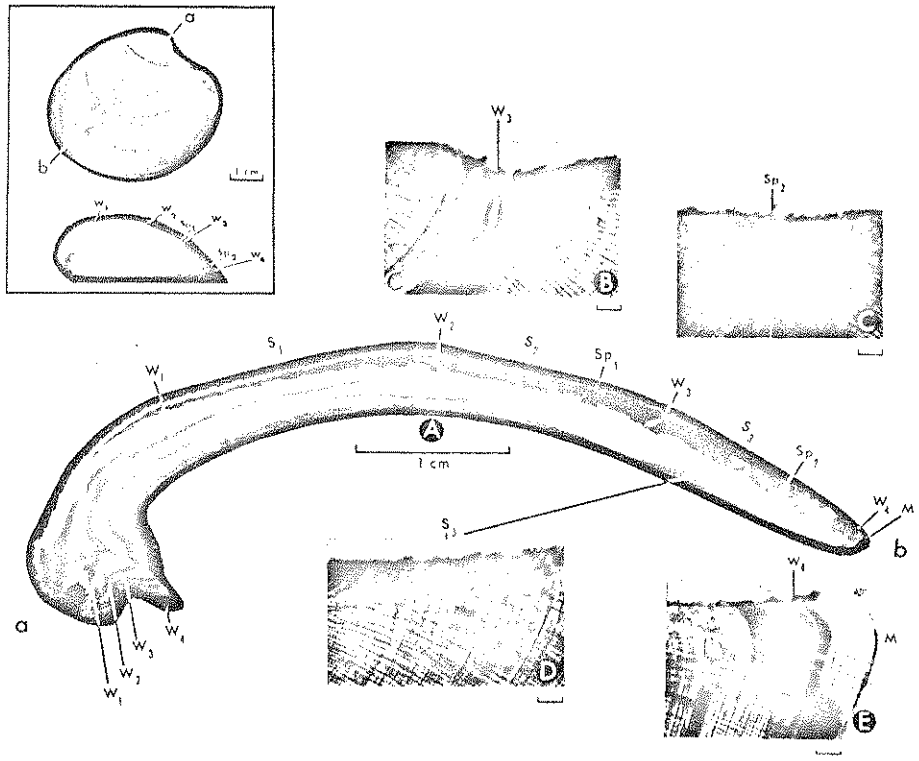

Figure 1. Illustration of bivalve shell sectioning technique. Box inset at upper left depicts axis of maximum growth (a–b) and external shell growth patterns of *Mercenaria mercenaria*. (A) Polished section along axis of maximum growth (a–b) of a specimen (depicted in inset) collected during the fourth winter (W_4). (S_1, S_2, S_3) Summer growth; (Sp_1, Sp_2) spawning breaks (see Chapter 7); (M) shell margin. (B–E) Microgrowth increments in specific shell regions of (A). Unlabeled scale bars: 100 μm.

coarsest grits early in this grinding sequence. Shell surfaces and all lapidary surfaces must be thoroughly cleaned after each grinding step to avoid contamination of the successively finer grits by the earlier coarse grits. As an alternative grinding procedure, one may utilize pressure-sensitive grinding discs (e.g., Buehler Ltd., AB carbimet special silicon carbide wet grinding papers) that are mounted on the lapidary wheels. These discs are available with the same size grits as the carborundum powders described above and should be used in the same sequential order (i.e., 120, 240, 320, etc.). If lapidary equipment is not available, grinding may be done directly on glass plates. For the final two grinding steps on glass, one should use 2000-grade, followed by 3000-grade, carborundum grits. No polishing (next step) is necessary if the surfaces are finely ground on glass in this manner.

1.4. Polishing

After sequential grinding (down to a 600-grade carborundum grit), surfaces are polished with 2000, followed by 3000, alumina powders [e.g., LINDE (Union Carbide, Crystal Products, Electronics Division) alumina powder abrasive] on a high-speed lapidary wheel covered with a polishing cloth (e.g., Buehler Ltd., AB microcloth No. 40-7208). A separate cloth must be used for each grit, and the lapidary wheel must be thoroughly rinsed after each polishing step.

1.5. Acid-Etching

After being polished, sections are etched by immersing them in a dilute solution of hydrochloric acid [1 part concentrated (38%) HCl to 100 parts distilled H_2O] for periods ranging from a few seconds to a few minutes. Optimal etching time will vary with the species examined and is related to shell structure, mineralogy, organic content, and state of preservation. The acid-etching process is a critical step in the technique, and it is recommended that a series of etching times be carried out to determine optimum etching periods for a particular specimen.

1.6. Washing and Drying

Immediately after the specimen is etched, the acid must be thoroughly rinsed from the sectioned surface. The section should be set aside to air-dry. Drying time can be reduced by spraying acetone on the etched surface.

1.7. Application of Acetone and Acetate

The etched shell surface is flooded with acetone, and a piece of sheet acetate (≈3 mm thick) is firmly applied to this surface. To eliminate air bubbles, one end of the piece of acetate should be placed at the edge of the cross section and then slowly lowered onto the meniscus of acetone, applying firm pressure. This procedure is similar to placing a cover slip onto a glass slide. A small weight should then be placed on top of the acetate to insure a firm contact of the epoxy block with the acetate. After the acetone has evaporated (20–30 min), the peel is removed from the cross section and, if desired, mounted onto a glass slide with Canada balsam or one of a number of synthetic resins prior to examination under the microscope.

Acetate-peel replicas may alternatively be prepared using extremely thin acetate. If this is done, the acetate should be placed on a *clean* piece

of glass. The shell cross section is then flooded with acetone and firmly applied onto the acetate. Once again, air-bubble formation may be minimized by placing one end of the cross section at the edge of the acetate and slowly lowering it onto the acetate, applying firm pressure throughout this process. A weight should be placed on top of the epoxy block. After 20–30 min, the acetate can be removed and should be immediately mounted between two glass slides to avoid curling and wrinkling.

2. Fractured Sections

Fractured sections of bivalve shells are extremely easy to prepare for scanning electron microscopic (SEM) examination. Shell valves should be thoroughly dried prior to fracturing; placement of the valves in a desiccator is advisable for shells from which soft tissues have recently been removed. Once dry, the shells may be fractured (i.e., broken) along the desired axis. For fracturing, it is generally advisable to place the shell on the edge of a solid object (e.g., a firm table) with the desired transect superimposed directly over the edge (of the table) itself. Firm pressure may then be applied to the unsupported portion of the shell extending beyond the table edge, until the shell breaks. It is important to break the shell away from any shell surface that is of interest. For example, if one is interested in examining the periostracum of a given shell valve, the shell should be placed on the edge of the object (table), with the periostracum side of the valve up; pressure is then applied downward so that the break itself is away from the periostracum. After being fractured, the shell fragment may be mounted on a standard SEM stub. Care should be taken to ensure electrical conductivity between the specimen and the stub; the use of silver paint or graphite-containing solutions (e.g., DAG 154, Ted Pella, Inc., Cat. No. 1603-16) as mounting media generally adequately provides such conductivity. Clean compressed air should be used to remove all contaminants from the surface of the fractured section. Finally, the specimon should be coated (under vacuum) with gold–palladium or a combination of gold and carbon.

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Reference

Rhoads, D. C., and Pannella, G., 1970, The use of molluscan shell growth patterns in ecology and paleoecology, *Lethaia* **3**:143–161.