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Preparation of Acetate Peels of Valves from the Ocean Quahog, *Arctica islandica*, for Age Determinations

John W. Ropes

U.S. DEPARTMENT OF COMMERCE
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National Marine Fisheries Service

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ABSTRACT

Techniques are described for preparing acetate peels of sectioned valves of ocean quahogs, *Arctica islandica*, for age determinations. The respective sequence of preparation begins by sectioning left valves oriented to include a single hinge tooth, bleaching to remove the heavy periostracum, embedding the valves in an epoxy resin, grinding and polishing the embedments to a high luster, etching the exposed cut valve surfaces, and applying sheet acetate with acetone. Annuli are clearly defined relative to growth increments in the peel preparations for all sizes and ages of ocean quahogs.

INTRODUCTION

Bivalve molluscs have historically been aged by examining the external valve surfaces for dark growth "rings" or "bands" that form as an annual periodic event (Weymouth 1923; Stevenson and Dickie 1954; Wilbur and Owen 1964; Merrill et al. 1966; Feder and Paul 1974). Ring formation has been associated with factors having probable effect on the metabolism of growth, such as extrinsic environmental conditions (temperature) or intrinsic conditions (spawning). In applying the method to some species, however, conflicting or incomplete age estimates may be obtained. This is particularly true for the ocean quahog, *Arctica islandica* Linné, a species with a potentially very long life history (Thompson et al. 1980a). Small ocean quahogs (<60 mm in shell length) often exhibit definite external rings useful in measuring growth and determining age. Some of the rings formed in the earliest years of the life of a large ocean quahog may be discernible, but those near the ventral valve margin usually become crowded together, and often the overall periostracum is black in color. Separation of the rings is impossible, even under magnification, and erosion of the shell surface in the umbonal area often obliterates the youngest rings.

In some bivalve species, internal narrow dark growth lines (hereafter termed annuli) alternating with broad light growth-increment deposits are seen in the broken edges of valves. The annuli have continuity with rings on the external valve surface. Development of diamond-impregnated saw blade equipment has greatly facilitated sectioning bivalve shells in a directed manner for an examination of the accretion of shell layers from the beginning of their formation at the umbo to the ventral margin. Although sectioning the valve is a time-consuming procedure, it permits an examination of the internal depositional features. These tend to be much less affected by destructive external environmental conditions impacting on the valve surfaces and, thus, a record of growth is preserved intact. Annual microstructural deposits may also occur in other parts of the shell that can be prepared for examination by fairly rapid methods, e.g., the chondrophores of the surf clam, *Spisula solidissima* (Dillwyn) (Ropes and O'Brien 1979).

Simply cutting the valves of a bivalve may not expose well-defined annuli, due to variations in the microstructure of the shell deposits. Such is the case for ocean quahogs. Kummel and Raup (1965) developed techniques for fossil bivalves involving preparation of the cut valve surfaces and transfer of the microstructural details onto sheet acetate.

The objectives in the development of procedures for ocean quahogs were that age/growth characteristics be determined precisely and efficiently. Assessment surveys of the population can provide large numbers of specimens for analysis, from 1,500-2,500 per survey. This paper describes procedures developed at the Northeast Fisheries Center for the preparation of acetate peels of sectioned bivalves. It is the only known technique at present for accurately determining the age of ocean quahogs, and more clearly exposes annuli in younger specimens than examination of the external valve surface. It also has potential application for other difficult-to-age bivalves.

PROCEDURES

Sample information is recorded for each ocean quahog preliminary to preparing the valves for aging. A sequential code number is inscribed on each valve of a pair, single valve, or piece of a valve. Measurements of length (the longest antero-posterior dimension), height (the deepest dorso-ventral dimension), width (the widest lateral dimension), and dry weight are made of paired, whole valves to 0.1 mm with vernier calipers, and weight to 0.1 g. Figure 1 shows the features of an ocean quahog valve for orientation purposes in measuring and sectioning operations.

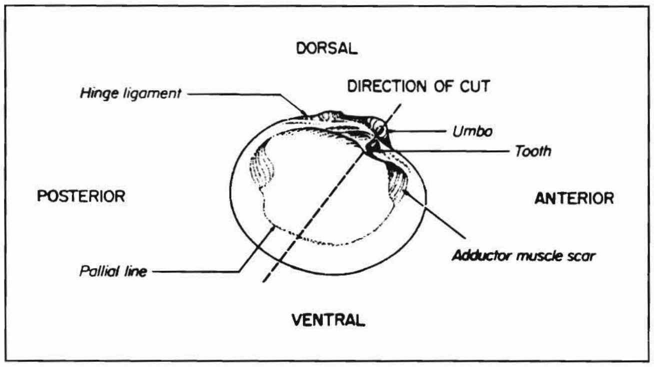

Figure 1—Sketch of internal valve features and direction of cut (dashed line) to completely section the left valve of an ocean quahog, *Arctica islandica*. The anterior portion of the valve is retained for subsequent processing.

The left valve of a pair is selected for sectioning, since it has a single prominent tooth in the hinge. The tooth contains annuli for exposure during the sectioning operation of the valve. Each valve is marked on the ventral margin at a point from the posterior end equal to one-third of the valve length. This orients sectioning through the umbo and parallel to the broadest tooth surface.

The valve is fastened firmly to the adjustable arm holder of the saw unit by a sticky, putty-like material (Slab-Stick) with its concave, inner surfaces toward a diamond-impregnated saw blade (Fig. 2, Table 1). The valve is oriented with the tooth toward the front of the saw machine and is positioned to cut from the mark on the ventral margin through the middle of the tooth, or immediately beside the posterior edge of the tooth. This latter orientation is necessary for small valves, since the small-sized tooth would be destroyed by the cut of the saw blade. Upon nearing completion of the cut through the valve, thumb pressure applied beneath the adjustable arm holder lessens the weight of the valve, and avoids breakage and saw blade damage. This procedure minimizes such problems by focusing the operator's attention on the cutting action of the saw. The anterior portion of the valve is saved for later treatment. About 7-8 medium sized (60-70 mm long) or 3-4 large sized (100+ mm) *Arctica* can be cut per hour at 300 rpm of the saw blade. No more than the heaviest weight (150 g) provided for the saw machine is used on the adjustable arm for large clams; the medium weight (75 g) for medium clams; and the lightest weight (25 g) for small clams. Excessive weight is avoided, since this can warp the blade or break the valve.

Aragonitic granules (a form of calcium carbonate) in the periostracum of ocean quahogs dissolve during a later etching step, leaving voids at the edge of valve peel preparations that distort the peel image (Ropes et al. 1984). This covering is therefore removed by immersing the sectioned cut surfaces in a shallow solution of full-strength household bleach for a few hours (Table 1). Each valve is periodically inspected and removed as soon as the periostracum is dissolved away. A rinse in tap water completes this step. Precautionary procedures for minimizing the destruction of ultrastruc-

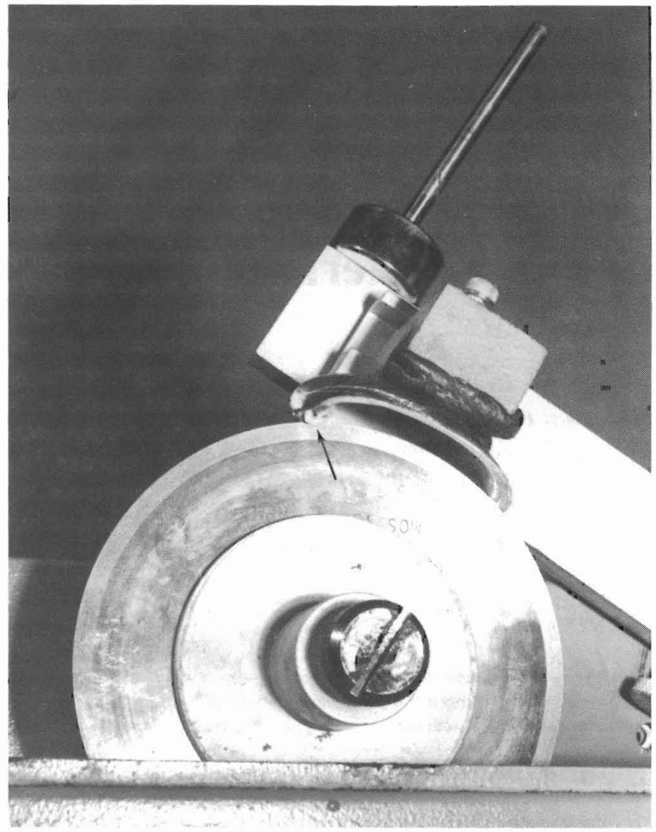

Figure 2—Close-up view of the left valve of an ocean quahog, *Arctica islandica*, fastened to the adjustable arm of the saw unit and oriented with the tooth (arrow) beside the diamond blade.

Table 1—Sources of apparatus and materials.

Source	Apparatus or material
Buehler Ltd. 41 Waukegan Road P.O. Box 1 Lake Bluff, IL 60044	Isomet Low Speed Saw Diamond Wafering Blades (4″ & 5″ dia., high concentration)
Commercial Plastics & Supply Corp. 352 McGrath Highway Sommerville, MA 02143	Di-acetate sheets 19×24×0.005″ thick
Miller Stephenson Chemical Co., Inc. P.O. Box 950 Danbury, CT 06810	Epon 815 and DTA hardener
Pigment Dispersions, Inc. 54 Kellogg Court P.O. Box 412 Edison, NJ 08818	#2301 blue pigment
Raytech Industries, Inc. P.O. Box 6 Stafford Springs, CT 06075	Hustler Vibrating Lap 15″ dia. Slab-Stick Ray-Tilt 6″ Gem Maker Grinding Machine
Local markets	Bleach (sodium hypochlorite ca. 5.25%) Norton, Tufbak, Durite, Waterproof paper, Closekote, Silicon Carbide

tural details by extended exposure of molluscan shells to bleach have been outlined by Carriker (1979).

Some grinding of the cut surfaces is advisable before further processing, if saw marks or other unwanted blemishes are evident, or if the saw cut was not close to the tooth. Careful handling is important in this step to minimize chipping of the cut valve edge. Carbide paper of two successively finer grits (240 and 400) is used for grinding and water is used on the paper to wash away particles (Table 1). About 15 min per specimen may be spent in this grinding operation. After thoroughly rinsing the valves in tap water, they are allowed to dry.

An epoxy resin is used for support of the valves during subsequent polishing operations (Table 1). The resin, hardener, and a colored pigment are carefully but thoroughly mixed (ca. 1 min), avoiding the introduction of bubbles, and in small amounts (50-75 mL) to minimize heat due to an exothermic reaction between the resin and hardener. The pigment added to the clear epoxy serves as a background, contrasting color with the white valves in a later grinding operation. The mixed epoxy is poured into plastic- or paraffin-coated molds to a depth of about ½ cm. When lowering a valve into the epoxy, the cut surface is pressed down onto the mold bottom to force out bubbles. Some bubbles may adhere to the valve surfaces, and if they are not removed may distort the acetate peel impression. These can be dislodged with the stirring stick used to mix the epoxy, but subjecting the set-up to the pressure of a vacuum chamber for an hour usually evacuates bubbles that may not be easily detected. After an overnight hardening period, the embedded valves are removed from the molds.

Three successively finer grits (240, 400, and 600) of carbide paper are then used to obtain a flat, smooth surface on the embedded cut surfaces of the valves. Most of the grinding is done with the 240 grit paper to remove epoxy and to expose the broadest area of the tooth; the finer grits (400 and 600) are used to minimize scratches from the coarser grit papers. Hand grinding tends to produce optimal results, because frequent inspection can be made of the progress of grinding; however, a grinding machine has proven to be faster (Table 1). Usually only the 240 grit is used on the machine to remove excess epoxy, followed by hand grinding to remove the circular and deep scratches that may have been produced by the machine. About 15-30 min per specimen may be spent exposing the tooth and valve surfaces. The pigment in the epoxy helps in determining the desired level of exposure of the cut surface. This initial grinding prepares the embedded valves for the final polishing steps.

A blemish-free, high-gloss surface on the cut valve surfaces is required and is produced on a vibrating lap machine in two steps (Table 1). In the first step, the pad lining the bottom of an attachable pan is flooded with tap water and drained. After clamping the pan on the machine, the pad is charged with about one teaspoon of #2 medium grit powder, the specimen is placed on the pad, and the machine is turned on. During a 1-2 hour period, the specimen is occasionally removed, rinsed off in tap water, damp-dried, and inspected for a moderate sheen. In the second step, the specimen is transferred to a similar pan flooded with water, but the pad is charged with fine grit (#3) powder. The valves are polished an additional 1-2 hours; more grit powder added after one hour hastens polishing. A final high gloss is most evident on the cut valve surface, but less so on the epoxy surface. A thorough washing in tap water removes traces of grit.

The polished block face is then placed in or flooded with a 1% HCl solution for one minute to etch the valve surfaces and rinsed in tap water, taking care not to damage the etched surfaces. After drying the block, code numbers for each valve are scribed with a carbide-tipped pen into the epoxy near the cut valve surfaces. These numbers are automatically transcribed onto the acetate peel during the next step.

Acetate peels are made by supporting the etched block with the surfaces uppermost and level. An acetate sheet is laid over the surface and a binder clip is used to hold it at one end (Table 1). The other end of the sheet is then lifted and acetone is pipetted onto the specimen while lowering the sheet. Acetone must flood completely between the sheet and block surface without bubbles for a successful peel. After draining of excess acetone, the whole block is turned over, laid on a pad of paper, and pressed for about one minute. After a one-hour drying period, the acetate is peeled off.

The peels are sandwiched between glass slides for examination under a light compound microscope, although the image may also be projected onto the screen of a fish-scale microprojector. Additional peels can be made by simply applying a new sheet of acetate and acetone to the specimen. A sheet of acetate left on the block protects the specimen from damage.

Processing-time economies

Sectioning the valves before embedding them is a departure from the technique described by Kennish et al. (1980), since it permits more certain orientation of a valve over the saw blade. It also speeds sectioning because there is no epoxy to cut through. Kennish et al. (1980) warned that unembedded valves may be fractured. Such damage to ocean quahogs was minimized by careful handling of the fairly robust valves. Individuals as small as 10 mm in shell length have been successfully prepared for aging.

Other modifications economize on time and materials, since there is a need to be efficient in the preparation process. Two saws have been used during the sectioning step. Unembedded, sectioned valves were polished and etched in an attempt to eliminate the embedding step, but the peels were distorted by acetate melting over the valve edges. This resulted in incomplete peel images at the valve edges. To further speed the process, groups of valves are placed in the bleach solution. Sectioned valves are lowered edge-first into a shallow depth of epoxy rather than embedding a whole valve. This permits grouping more than one valve (up to 3-4) in a single mold. The group of embedded valves is also more easily handled during the later grinding/polishing, etching, and peel steps.

Identity of annuli

Acetate peels of many marked ocean quahogs recovered in subsequent years have been prepared, since these individuals add significant support to the hypothesis that annuli are accreted annually and make possible the identification of annuli (Thompson et al. 1980a, b; Jones 1980; Bennett et al. 1982; Turekian et al. 1982; Murawski et al. 1982; Ropes et al. 1984). A large ocean quahog valve marked in 1978 and recovered in 1983 is shown in Figures 3a and b. The black periostracum has been peeled away in the marked area to reveal the lack of discernible growth after the clam had been marked (measurement indicated growth of only 0.7 mm). External rings were not evident in the periostracal layer or annuli beneath it. In contrast, a small ocean quahog valve marked and recaptured during the same years displayed visible external rings in the periostracal layer and substantial growth (14.4 mm) after marking (Fig. 4a).

A photomicrograph of the acetate peel produced by our method of sectioning, embedding, polishing, and etching the ventral valve margin of the large specimen shows an indentation from the mark-

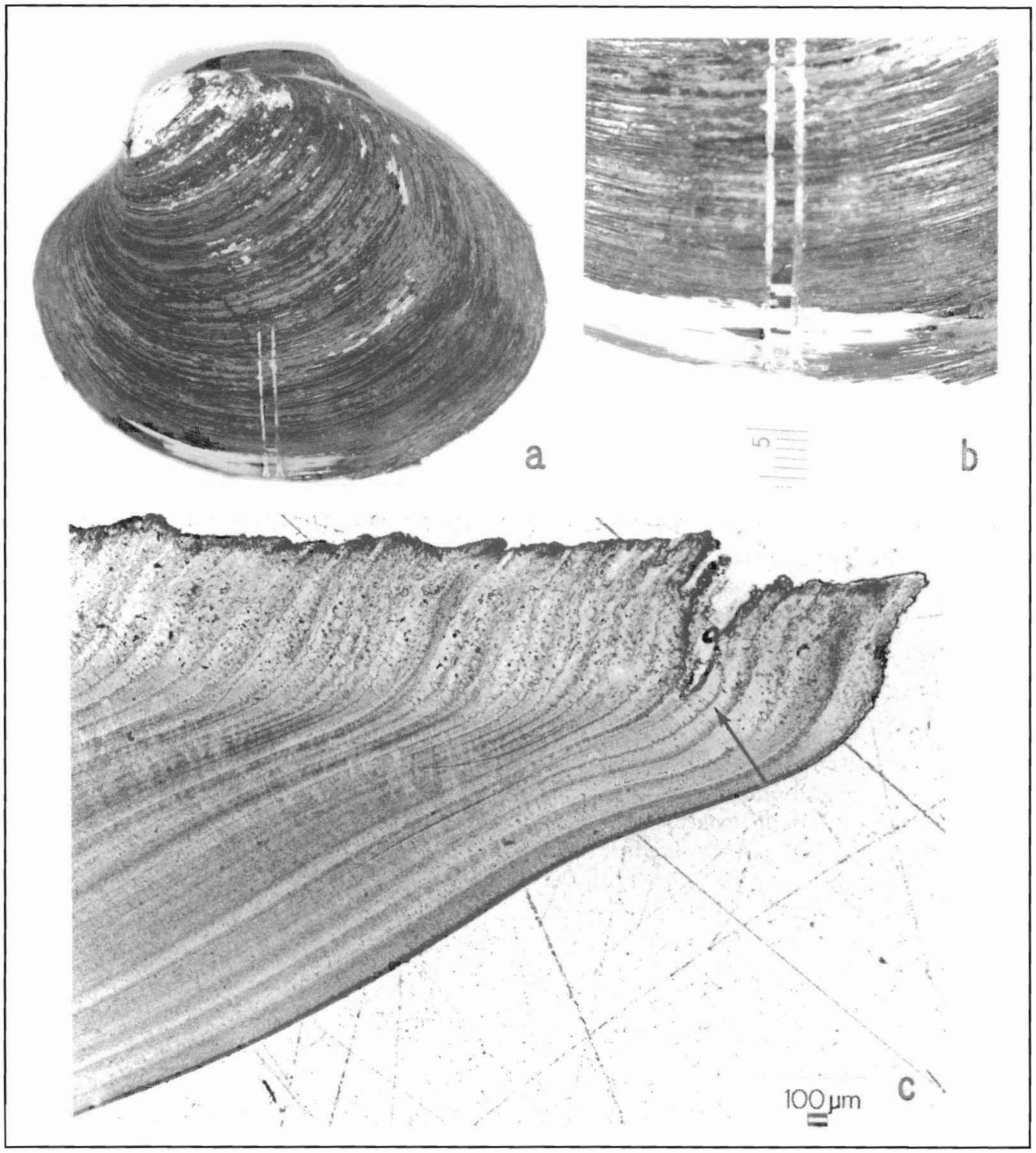

Figure 3—a) A 110-mm shell length ocean quahog, *Arctica islandica*, marked in 1978 and recaptured in 1983. b) Enlargement of the paired parallel notch marks made at the ventral valve margin. Note that growth (estimated to be 0.7 mm) after notching is barely visible. A millimeter scale was photographed with the valve. c) Replicate peel photomicrograph of the sectioned ventral margin showing the annulus formed soon after marking (arrow) and four annuli formed thereafter. A metric scale is included.

ing operation, the annulus formed soon after it was marked, and four annuli thereafter (Fig. 3a). This clam and the small individual were captured before the annuli for 1983 had formed. Annuli appear as dark wavy lines at the valve edge, but become more condensed thin lines back into the valve, conditions typical for large, old clams. The annuli were identified as prismatic microstructures in Scanning Electron Microscope (SEM) examinations (Ropes et al. 1984). Growth increments have a light granular appearance under a light microscope consistent with a predominantly homogenous microstructure of ocean quahog shells. The very fine microstructural units are not evident under a light microscope.

The same features seen in the peel of the large ocean quahog occur in the peel of the small individual, except that the growth increments between annuli are more extensive and the annuli are typically nonwavy condensed lines (Fig. 4c). A peel of the hinge tooth shows the same pattern of condensed annuli and growth increments for the small individual (Fig. 4b).

SUMMARY

Despite procedural modifications, the production of acetate peels remains labor-intensive and time-consuming. It is the only known method of producing accurate age data for large ocean quahogs, and selected samples have provided significant information on the age/growth phenomena of the ocean quahog. *Arctica* has been characterized as a slow growing, long-lived bivalve [a 225-year-old individual may be the oldest recorded bivalve (Ropes and Murawski 1983)] and it is not uncommon to capture individuals 100 years old. The frequent occurrence of old individuals complicates application of the method on a population-wide basis.

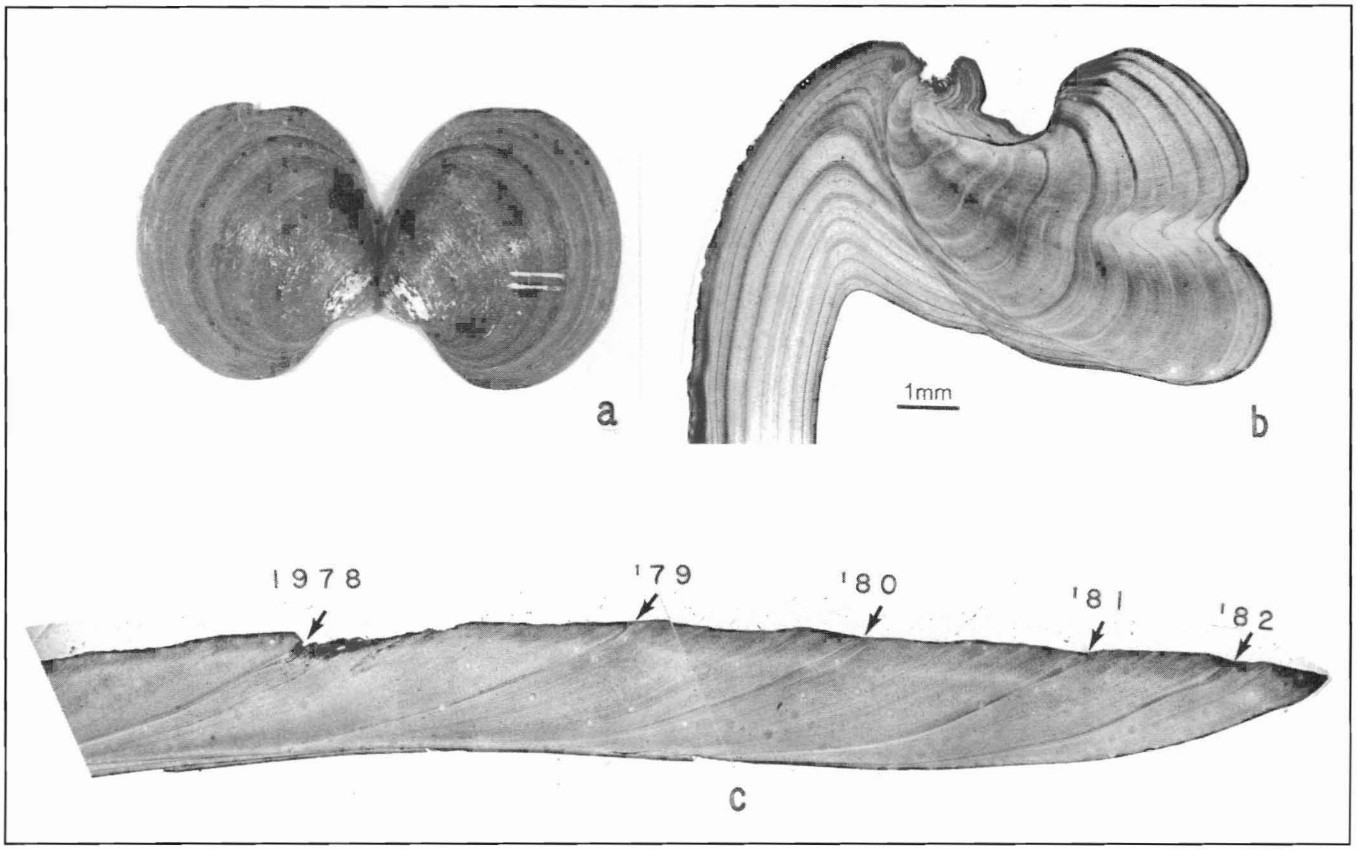

Figure 4—a) A 62-mm shell length ocean quahog, *Arctica islandica*, marked in 1978 and recaptured in 1983. Paired parallel notches clearly delimit growth (estimated to be 14.4 mm) after marking. b) Eleven annuli are shown in the photomicrograph of the clam's hinge tooth. These corresponded to the same number of annuli found in the valve. c) Replicate peel photomicrograph of the sectioned ventral valve margin showing an annulus that had formed soon after marking and four annuli formed thereafter. Each annulus is identified by the year of its formation. A millimeter scale applies to peel replicate photomicrographs b and c.

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