

HARD CLAM, *MERCENARIA MERCENARIA*: SHELL GROWTH PATTERNS IN CHESAPEAKE BAY^{1, 2}

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ABSTRACT

Dark bands in the middle homogenous layer of *Mercenaria mercenaria* shells, formed each summer and early fall in lower Chesapeake Bay experimental and wild populations, were used to determine age. Distinct growth cessation marks caused by low winter water temperatures were present in some annual increments, but were not formed each year by each individual. This was due primarily to differences among age groups in seasonal band formation. *Mercenaria mercenaria* younger than 8 years tended to form light bands in fall and spring, which were bisected by distinct winter growth cessation marks. Older individuals tended to form light bands only in spring; thus, winter growth cessation marks were masked by dark bands deposited from summer through winter. These results differ from *M. mercenaria* shell growth patterns found elsewhere along its range, suggesting that time of annulus formation varies with latitude.

One microgrowth increment in the prismatic layer was formed during each solar day of activity (growth). From a 106-day monitored growth experiment in summer 1980, the slope of the regression describing the relationship between the number of increments formed (Y) and days (X) was not significantly different from 1.00 ($t_s = 1.23$, $P > 0.20$, $r = 0.98$). Inactive periods, represented by growth cessation marks, became longer and/or more frequent with increasing age and length of monitored growth periods. Both factors, increasing age and length of monitored growth periods, contributed to decreased increment-to-day ratios.

There has been considerable research on the periodicity of line, band, zone, and increment formation in bivalve shell microstructure since Barker's (1964) initial description (see Lutz and Rhoads 1980). Annual shell increments have been identified in shells of many species, including *Arctica islandica* (Thompson et al. 1980), *Mya arenaria* (MacDonald and Thomas 1980), *Spisula solidissima* (Jones et al. 1978), and *Geukensia demissa* (Lutz 1977; Lutz and Rhoads 1978⁵; Lutz and Castagna 1980). In shells of the hard clam, *Mercenaria mercenaria* (Linnaeus, 1758), annual increments have been described in two distinct ways: 1) Regions of narrow and wide microgrowth increments in the outer prismatic layer resulting from seasonal changes in growth rate (Pannella and MacClintock 1968; Rhoads and Pannella 1970) and 2) a single pair of translucent and opaque

zones in the middle homogenous layer (as viewed in thin radial section; Clark 1979). However, these definitions are not mutually exclusive, since translucent zones are associated with narrower microgrowth increments (or slower growth rates) than opaque zones (Clark 1979).

The season of slow shell growth by *M. mercenaria* varies along its latitudinal range (Gulf of St. Lawrence to Gulf of Mexico; Franz and Merrill 1980). In the north-central part of its range (Connecticut, Massachusetts, and New Jersey), reduced growth rates and growth cessations occur during winter, microgrowth increments being between 2 and 100 times narrower than those formed in summer (Pannella and MacClintock 1968; Rhoads and Pannella 1970; Kenish and Olsson 1975). Conversely, *M. mercenaria* in the southern part of its range (Georgia) grow slowly in summer and early fall when translucent zone formation occurs (Clark 1979). *Mercenaria mercenaria* in Georgia may also grow throughout winter, since no winter growth cessation marks have been observed in shell microstructure (Clark 1979). Thus, latitudinal variation may preclude the universal application of defined annual shell increments to all populations along the range of the hard clam. Shell growth patterns of local populations must be analyzed to determine its unique features.

There have been no previous studies of microstructural shell growth patterns of *M. mercenaria* in lower Chesapeake Bay. Hard clams used in this study to

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⁵Lutz, R. A., and D. C. Rhoads. 1978. Shell structure of the Atlantic ribbed mussel, *Geukensia demissa* (Dillwyn): A re-evaluation. Bull. Am. Malacol. Union 44th Annu. Meet., p. 13-17.

determine annual shell increments were from experimental growth lots established by Loesch and Haven (1973). Monitored growth periods of hard clams in these lots, as long as 13 yr, are the longest of any bivalve shell growth study in the literature.

MATERIALS AND METHODS

Sources of *Mercenaria mercenaria*

Mercenaria mercenaria were obtained from three sources for use in this study: 1) Four long-term experimental growth lots, 2) two short-term experimental growth lots, and 3) natural population or wild stock. Long-term studies lasting a maximum of 13 yr were initiated in 1967 and 1969 at four subtidal locations in the lower James and York Rivers [Table 1; see also Loesch and Haven (1973) for a description of long-term growth lots]. Hard clams in each group were numbered individually (using an indelible ink pen) and measured (shell length—greatest distance along the anterior-posterior axis—to the nearest 0.1 mm) prior to placement directly in the substrate by scuba-equipped divers. As many hard clams as possible were retrieved, measured, and replanted at the lot location each fall through 1972. From fall 1972 to the dates of final collection between 1976 and 1980, each group remained in the substrate continuously. Shell height (greatest distance from umbo to ventral edge) was not measured from 1967 to 1972. After final collection, however, shell length measurements obtained each fall were used to identify growth rings on the shell exterior of each hard clam. Shell height was measured at each of these growth rings to yield a size-time relationship along the height axis, along which valves were cut for microstructural analyses.

Short-term growth studies began on 16 October

TABLE 1.—Experimental groups of *Mercenaria mercenaria* used in long-term and short-term studies.

Study and lot no.	Location	Date of lot establishment	N	Date of final collection
Long-term study:				
I	York River	9/67	16	12/76
			1	6/80
			1	7/80
II	York River	9/67	15	8/76
			10	1/78
			8	2/78
			26	7/79
XI	James River	1/69	26	7/79
XIV	York River	9/69	6	8/76
			6	1/78
Short-term study:				
T series	York River	10/16/79	71	Monthly to 6/27/81
TI series	York River	5/30/80	12	Monthly to 9/13/80
Total			172	

1979 and continued for 20 mo (Table 1). Age 2+ *M. mercenaria* were obtained from the Virginia Institute of Marine Science hatchery on Virginia's Eastern Shore (Castagna and Kraeuter 1977). Each individual was numbered, measured (shell length and height), and transplanted to a subtidal location in the York River. Collections of four hard clams each were made from this T series group at approximately monthly intervals. Shell growth did not resume until April 1980, probably due to the combined effects of salinity difference between the Eastern Shore (28-30 ppt) and York River (16-18 ppt) and low winter water temperatures. Because of this, the exact date of growth resumption in spring 1980 was unknown.

The TI series was composed of T series hard clams in which a growth cessation mark was induced in spring 1980 (Table 1). This was used as a baseline for determining the periodicity of formation of prismatic microgrowth increments. Growth cessation marks in shell microstructure were induced by the thermal shock method of Richardson et al. (1979). On 29 May 1980, 16 T series hard clams were collected, measured, renumbered, and placed in a moist incubator at 4°C for 24 h to disrupt shell growth. TI series hard clams were replanted on 30 May 1980 in a segregated area of the T series location. Three TI series hard clams were collected and measured on 22 June, 18 July, 8 August, and 13 September 1980.

Mercenaria mercenaria from the natural population of lower Chesapeake Bay and its tributaries ($N = 24$) were collected during winter, spring, and summer of 1978 and 1980. Shell height and length of each hard clam were measured.

Preparation of Acetate Peels

Acetate peels of polished and etched radial shell surfaces were prepared from single valves of each experimental and wild hard clam according to the methods of Stewart and Taylor (1965) and Pannella and MacClintock (1968). Valves were cleaned and air-dried for several days prior to being embedded in liquid casting plastic⁶ and cut from ventral edge to umbo along the height axis with a geological saw. One of the sectional surfaces was ground and polished with optical quality grits and cerium oxide on glass plates and a cloth-covered disc polisher. Polished surfaces were etched in either 1% or 5% HCl for 20-60 s and dried completely. Clear acetate sheets (0.003-in thick) were carefully melted on each etched

⁶American Handicrafts, Inc., Fort Worth, Texas (reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA).

surface with acetone and air-dried for at least 1 h, after which they were stored between glass microscope slides.

Analysis of Shell Microstructure— Terminology and Methods

Clark (1979) described a series of translucent and opaque zones in the middle homogenous layer of thin sections of *M. mercenaria* shells. These zones were associated with narrow and wide prismatic microgrowth increments, respectively (Table 2). In this study, acetate peels of etched radial sections, rather than thin sections, were used. Regions of low and high light transmittance through acetate peels corresponded exactly with "dark" and "light bands," respectively, in the middle homogenous layer of polished shell sections (Table 2; see Figure 2). For convenience, we refer to regions of low and high light transmittance through acetate peels as dark and light bands. Clark's description of translucent zones in thin sections could also be correctly applied to dark bands in acetate peels, since both were associated with narrow microgrowth increments. However, regions of the middle homogenous layer associated with narrow increments appear translucent in thin sections, but optically dense or "dark" in acetate peels. Conversely, opaque zones in thin sections appear transparent or "light" on acetate peels (Table 2). Since Clark's terminology from analyses of thin sections does not strictly apply to middle homogenous layer growth patterns observed on acetate peels, we have used the new terms "dark" and "light bands," as outlined in Table 2. However, translucent zones and dark bands, and opaque zones and light bands describe the same growth pattern in shell microstructure.

Acetate peels were analyzed on a compound microscope at 100× magnification with nonpolarized light. Known annual shell increments formed between 1967 and 1972 by experimental hard clams in lots I, II, XI, and XIV were analyzed for annually produced patterns. Similarly, total shell increments

deposited between 1972 and the date of final collection by each experimental hard clam in the four lots should contain the same number of annual increments as there were years in the period. Annual increments were defined primarily in the middle homogenous layer due to the simplicity of its growth pattern (dark and light bands) compared with the outer prismatic layer (microgrowth increments). Band color at the shell margin was catalogued by season of collection in both experimental and wild hard clams to determine time of year of dark and light band formation. To increase the number of fall observations, band color was also observed dorsal (toward the umbo) to each disturbance mark in shell microstructure of long-term experimental hard clams caused by measurements in 1967-72. Observations of band color in each season were catalogued by three age groups defined by Kennish (1980): Young—under 3 yr of age; mature—3 to 8 yr; old—over 8 yr.

Microgrowth increments in the prismatic layer (their average width and number) were used to describe bands in the middle homogenous layer. Individual increments were traced through the prismatic to the middle layer to identify single increments corresponding to the dorsal (toward the umbo) and ventral (toward the shell margin) surfaces of each band. Band width was measured along the surface of maximum growth (SMG) in the prismatic layer (Pannella and MacClintock 1968) using an ocular reticle with an estimated accuracy of ± 1 reticle unit ($10.8 \mu\text{m}$ at 100×). Microgrowth increment counts were made only in shell regions bracketed by growth disturbance marks of known formation time or one growth disturbance mark and the shell margin (collection date). This allowed determination of the periodicity of increment formation. All microgrowth increment counts in bands or annual shell increments were averages of three trials. Guidelines suggested by Crabtree et al. (1979/1980) were used to distinguish and count microgrowth increments. Least squares linear regressions (Sokal and Rohlf 1969) of increment counts on days in monitored growth

TABLE 2.—Terminology used to describe growth patterns in the middle homogenous layer of *Mercenaria mercenaria*.

Reference	Technique	Light	Descriptive terms for pattern associated with:	
			Narrow microgrowth increments	Wide microgrowth increments
Clark (1979)	Thin section	Transmitted	Translucent zone	Opaque zone
This study	Acetate peel	Transmitted	Low light transmittance region	High light transmittance region
This study	Polished shell section	Reflected	Dark band	Light band

periods were used to support conclusions on the periodicity of increment formation. Comparisons of regression coefficients were done using either a t -test (t_s) if the comparison was between a calculated coefficient and its expected value, or an F -test (F_s) if the comparison was between two calculated coefficients (Sokal and Rohlf 1969). To ascertain effects of age on number of increments formed in annual shell increments, microgrowth increments were counted between each pair of fall measurement disturbance marks (MDM) in long-term experimental hard clams (lot XI) formed 1 yr apart. Each count was divided by the number of solar days between measurements, yielding the percent agreement (Richardson et al. 1979) between increments and days. Data were pooled by absolute hard clam age.

RESULTS

Annual Shell Increments— Light and Dark Bands

The series of fall MDM divided the shell microstructure of 89 long-term experimental hard clams into 177 known years of shell growth formed between 1967 and 1972 (Table 3). A single dark band in the

TABLE 3.—Total numbers of known years of shell growth between 1967 and 1972 and dark bands in the middle homogenous layer observed in the known shell increments of long-term experimental *Mercenaria mercenaria*.

Lot no.	No. of clams analyzed	Total no. of known years for all clams ¹	Total no. of dark bands
I	18	35	35
II	33	43	43
XI	26	86	86
XIV	12	13	13
Totals	89	177	177

¹Many of the hard clams from each lot were added to it after the lot had been established. Because of this, the number of known years of shell growth for all hard clams in each lot is always less than the number of hard clams analyzed multiplied by the maximum number of known years of shell growth in each hard clam from 1967 to 1972 (5 yr).

middle homogenous layer had been formed within each known annual shell increment (see Figure 2). Furthermore, dark bands were located dorsal to the MDM, suggesting that they had been formed each summer. To confirm this observation, the number of complete summers from fall 1972 to the date of final collection in each hard clam was compared with the number of completed dark bands observed in shell microstructure formed in these periods (Table 4). Complete dark bands were defined as those which were not at the shell margin. Ninety-four percent (84/89) of the hard clams examined contained the same number of completed dark bands as there were complete summers, while the remaining 6% (5/89) formed one fewer dark band than years. This is regarded as the error estimate (6%) of this procedure for determining age of *M. mercenaria*.

Analysis of shell margin growth bands of all experimental and wild hard clams collected seasonally also revealed that dark band formation occurred during summer (Table 5; Fig. 1). The percentage of all ages of hard clams collected in summer which had a dark band at the shell margin (91%) was over twice that of hard clams collected in winter (40%). However, a significant proportion of hard clams collected in fall had a dark band at the shell margin (78%), indicating that the period of dark band formation also extended into fall.

Further examination of Figure 1 reveals differences among age groups in color of shell margin bands in fall and winter. The percentage of hard clams in all age groups with a dark band at the shell margin in summer ranged between 88 and 100% (Table 5; Fig. 1). However, in fall and winter, differences between age groups began to appear. In fall, 100% of old and 75% of mature hard clams had dark bands at the shell margin (Table 5; Fig. 1). The percentage with dark bands in winter declined in both age groups, but was still larger in old (44%) than mature (17%) hard clams. Thus, light band formation began sooner

TABLE 4.—Number of long-term experimental *Mercenaria mercenaria* with expected number of complete dark bands in the middle homogeneous layer in known years of shell growth between 1972 and dates of final collection.

Lot no.	Date of final collection	Complete summers	Expected no. of complete dark bands	No. of clams analyzed	No. of clams with expected no. of dark bands
II	8/76	1973-75	3	15	15
XIV	8/76	1973-75	3	6	5
I	12/76	1973-76	4	16	16
II	1/78	1973-77	5	10	8
XIV	1/78	1973-77	5	6	6
II	2/78	1973-77	5	8	8
XI	7/79	1973-78	6	26	24
I	6/80	1973-79	7	1	1
I	7/80	1973-79	7	1	1
Totals				89	84

(after the summer dark band was completed) in a greater percentage of mature than old hard clams. This could also have been due to a lack of growth by old hard clams in fall and winter, leaving the summer dark band at the shell margin.

Enlargements of acetate peels in Figure 2 further illustrate the time of dark band formation each year. Figure 2A-C form a representative summer-to-winter series of shell margin bands formed by mature (T and TI) hard clams. Dark bands were at the shell margin in hard clams collected in summer (Fig. 2A), while in hard clams collected in fall and winter, dark bands became separated from the margin by light

TABLE 5.— Summary of seasonal shell margin growth bands in long-term experimental and wild *Mercenaria mercenaria* (in summer, winter, and spring collections) and those dorsal to each fall measurement disturbance mark in 1968-72. Group collected in each season was subdivided by age according to Kennish (1980; see legend to Figure 1).

Season	Months	Age	N	Light band		Dark band	
				N	%	N	%
Spring	Mar.-Apr.	Mature	5	5	100	0	0
		Old	3	0	0	3	100
		Total	8	5	62	3	38
Summer	June-Sept.	Young	3	0	0	3	100
		Mature	18	1	6	17	94
		Old	34	4	12	30	88
Total		55	5	9	50	91	
Fall	Oct.-Nov.	Mature	139	35	25	104	75
		Old	17	0	0	17	100
		Total	156	35	22	121	78
Winter	Dec.-Feb.	Mature	6	5	83	1	17
		Old	39	22	56	17	44
		Total	45	27	60	18	40

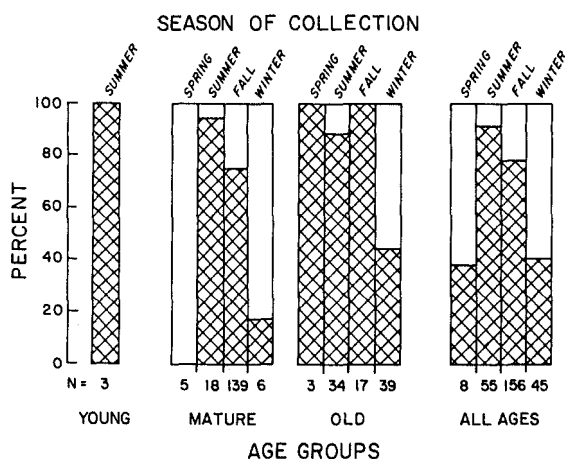
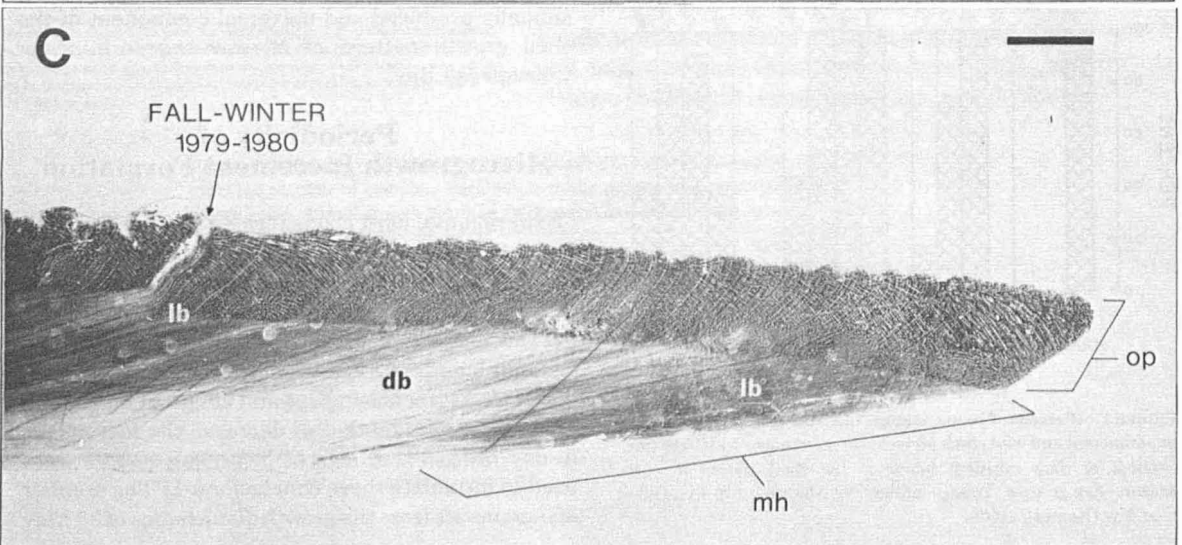
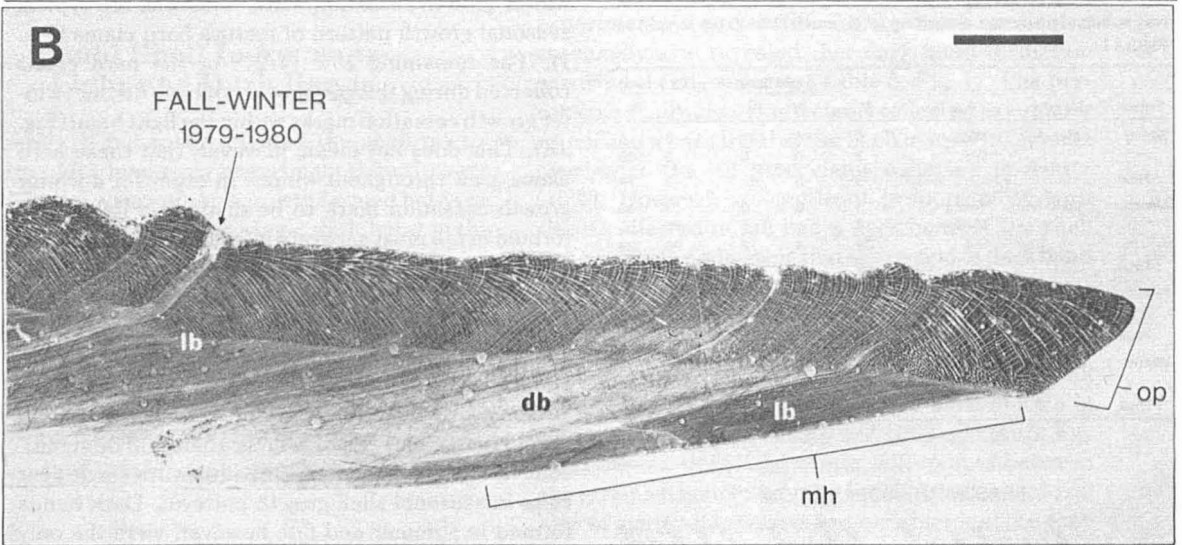
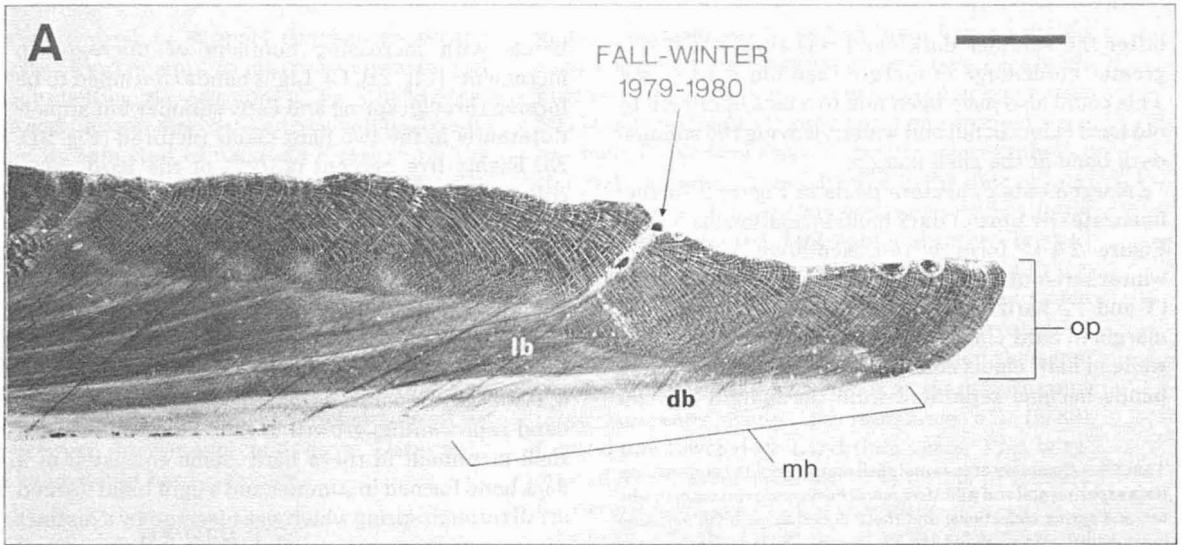


FIGURE 1.— Percent of young, mature, old, and all ages of long-term experimental and wild stock *Mercenaria mercenaria* with light (unshaded) or dark (shaded) bands at the shell margin in each season. Age groups: Young—under 3 yr, Mature—3 to 8 yr, Old—over 8 yr (Kennish 1980).

bands with increasing numbers of microgrowth increments (Fig. 2B, C). Light bands continued to be formed through spring and early summer but appear differently in the two hard clams pictured (Fig. 2D, E). Eighty-five percent (23/27) of the hard clams collected during or after winter 1980 (from December 1980 to June 1981) had a growth cessation mark within the shell margin light band formed during winter (Fig. 2D). This mark, termed a distinct winter growth cessation mark, was a thick microgrowth increment boundary in the light band with narrow microgrowth increments dorsal and ventral to it. It was also separated from the dark band by a light band representing growth in fall. Thus, one annual shell increment in these hard clams consisted of a dark band formed in summer and a light band formed in fall through spring which was bisected by a distinct winter growth cessation mark. This was the typical seasonal growth pattern of mature hard clams (Fig. 1). The remaining 15% (4/27) of the hard clams collected during this period did not have distinct winter growth cessation marks within the light band (Fig. 2E). This does not mean, however, that these hard clams grew throughout winter. In order for a winter growth cessation mark to be distinct, a light band formed in fall must separate it from the summer dark band. Consequently, lack of a distinct winter mark was more likely caused by lack of light band formation in fall. One annual increment in these hard clams consisted of a dark band formed in summer and fall and a light band formed in the following spring. This seasonal growth pattern was similar to that described for old hard clams (Fig. 1). Thus, there can be significant variation even among individuals in a single year class in seasonal shell growth patterns. Dark bands formed in summer and fall, however, were the only annually produced and universal component of the shell growth pattern of *M. mercenaria* in lower Chesapeake Bay.

Periodicity of Microgrowth Increment Formation

Experimental hard clams formed one microgrowth prismatic increment during each solar day of activity. Inactive periods, represented by growth cessation marks or thick organic lines in the prismatic layer, became longer and/or more frequent with increasing age and length of monitored growth periods. Thus, both factors (increasing age and length of monitored growth periods) tended to decrease the increment-to-day ratio. Three sets of increment counts were used to formulate these conclusions: 1) The number of increments from the growth disturbance of 30 May



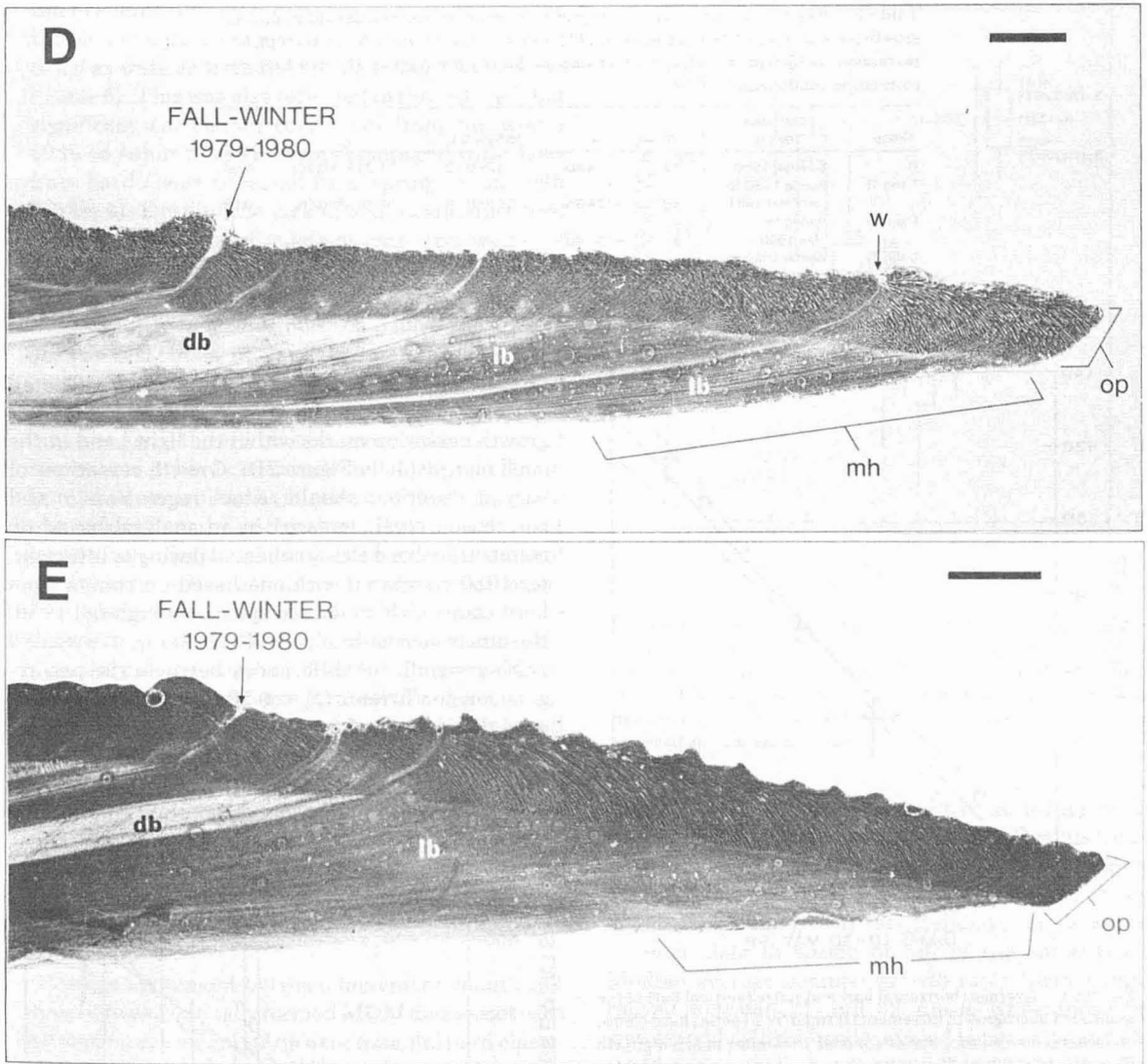


FIGURE 2.—Enlargements of acetate peels from five short-term experimental *Mercenaria mercenaria* showing shell growth from transplantation in October 1979 to each date of collection (shell margin). Growth disturbance due to transplantation is labelled fall-winter 1979-1980 in the outer prismatic layer (op) of each. Middle homogenous layer (mh) bands are labelled lb (light band) and db (dark band). Contrast in photographs is due to differences in transparency of portions of the peels. Light regions in photographs correspond to relatively opaque regions on peels, or those which appear dark in polished shell sections. Scale bars represent 1 mm and growth is to the right.

1980 to the shell margin in four collections of TI hard clams during summer 1980, 2) the number of increments from the growth disturbance caused by transplantation of short-term hard clams (from the Eastern Shore to the York River) on 16 October 1979 to the shell margin in hard clams collected from April 1980 (after growth had resumed) to June 1981, and 3) the number of increments between MDM formed 1 yr apart between 1969 and 1971 by hard clams in lot XI.

TI Series, From 30 May 1980

TI hard clams collected in summer 1980 had a strong tendency to form one increment each solar day (Table 6; Fig. 3). Regression of the number of increments formed on days since 30 May 1980 yielded a strong linear relationship ($F = 156.30$, $P < 0.001$) with a regression coefficient (b) not significantly different from 1.00 (Table 6). Consequently, TI hard clams tended to form one prismatic

TABLE 6.—Regression statistics for microgrowth increment counts on days in monitored growth periods of short-term experimental *Mercenaria mercenaria* $a = Y$ -intercept, $b =$ regression coefficient (slope); $t_s = t$ -test statistic for $H_0: \beta = 1.00$ vs. $H_1: \beta \neq 1.00$; $r =$ correlation coefficient.

Group	Collection period	N	a	$b \pm 95\% \text{ C.L.}^1$	t_s	r
TI	Summer 1980	12	-4.40	1.10 ± 0.19	1.23 ($P > 0.20$)	0.98
T and TI	Spring 1980 to summer 1981	58	-124.38	0.88 ± 0.13	-1.80 ($P > 0.05$)	0.88
T and TI	Spring to fall 1980	31	-197.48	1.14 ± 0.29	0.99 ($P > 0.20$)	0.87
T and TI	Winter 1980 to summer 1981	27	-48.60	0.74 ± 0.52	-1.04 ($P > 0.20$)	0.53

¹C.L. = confidence limits.

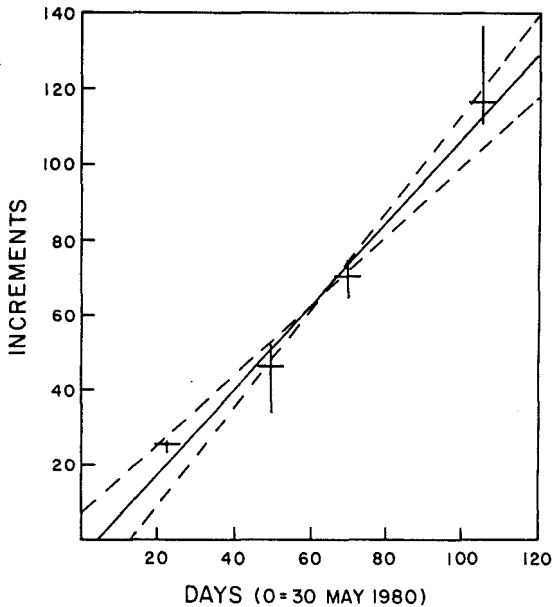


FIGURE 3.—Average (horizontal bar) and range (vertical bar) of the number of microgrowth increments formed by TI series hard clams, *Mercenaria mercenaria*, during summer 1980 after induced growth disturbance of 29-30 May 1980. Zero on abscissa equals 30 May 1980. Solid line = regression; dashed lines = $\pm 95\%$ confidence limits on regression coefficient (Table 6).

increment during each solar day for the 4 summer months.

T and TI Series, From 16 October 1979

T and TI hard clams collected from spring 1980 to summer 1981 also tended to form one increment for each solar day of activity. Regression analysis revealed a strong linear relationship between numbers of increments formed and days since 16 October 1979 ($F = 177.25$, $P < 0.001$; Table 6). Furthermore, the regression coefficient was not significantly different from 1.00 (Table 6). These statistics tend to

obscure the fact that 85% of hard clams collected during or after winter 1980 had distinct winter growth cessation marks within the light band at the shell margin (as in Figure 2D). Growth cessations of varying durations should reduce regression (b) and correlation coefficients (r) in an analysis based on counts from hard clams collected during or after winter 1980 compared with one based on counts from hard clams collected from spring through fall 1980. Results from such analyses (Table 6; Fig. 4) revealed neither significant differences between the two regression coefficients ($F_s = 0.50$, $P > 0.25$) or significant differences of both from 1.00. However, con-

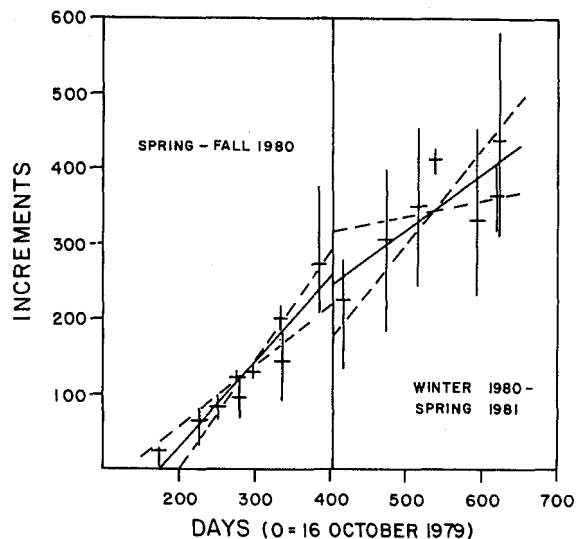


FIGURE 4.—Average and range (as in Figure 3) of the number of microgrowth increments formed by T and TI series hard clams, *Mercenaria mercenaria*, from fall 1979 to summer 1981 in hard clams collected after April 1980 to June 1981. Increments were counted from growth disturbance caused by transplantation in October 1979. Zero on abscissa equals 16 October 1979. Regression analyses based on 31 hard clams collected from spring to fall 1980 and 27 clams collected from winter 1980 to summer 1981. Solid and dashed lines as in Figure 3 (see also Table 6).

fidence limits on the regression coefficient from the winter 1980 to summer 1981 counts were almost twice as wide as from the spring to fall 1980 counts (Table 6). This was also reflected in the reduced, but significant correlation coefficient from the winter 1980 to summer 1981 counts compared with those from hard clams collected from spring to fall 1980 (Table 6). Despite the lack of significant statistical results, these data suggest that the ratio of increments to days was lower in hard clams collected during or after winter 1980 than in those collected from spring to fall 1980. This could have been due to growth cessations of varying durations in winter. However, growth cessations could also have occurred at anytime during the monitored growth period, and thus obscured the effects of winter on the number of increments in hard clams collected during or after it. Individual variability in numbers of days of growth was evident in the increasing range in increment counts from single collections with time. Chances of disturbances (such as storms, predation attempts, etc.) occurring in any season which could cause growth to cease in some hard clams would also increase with the length of monitored growth periods. Consequently, a one-to-one increment-to-day relationship only applied to short periods of monitored growth during favorable seasons, such as the TI hard clams discussed previously (Fig. 3). Prismatic microgrowth increments, however, each represented a solar day, despite the lack of one-to-one correspondence for long periods of monitored growth.

Lot XI, From 1969 to 1971

Percent agreement between increment counts and days between annually formed MDM decreased with increasing age of long-term experimental hard clams. Results of counts from lot XI hard clams age 3 to 10 in annual shell increments formed between 1969 and 1971 are shown in Figure 5. Results from other long-term experimental lots were similar. Consequently, experimental hard clams formed increments (were active) for fewer days each year with increasing age, indicating that growth cessations became more frequent, longer, or both.

Microgrowth Increment Widths, Seasonal Growth Rates

Average microgrowth increment widths associated with dark bands were generally smaller than those associated with light bands in all long-term and short-term experimental hard clams. The distribution of average increment widths formed between

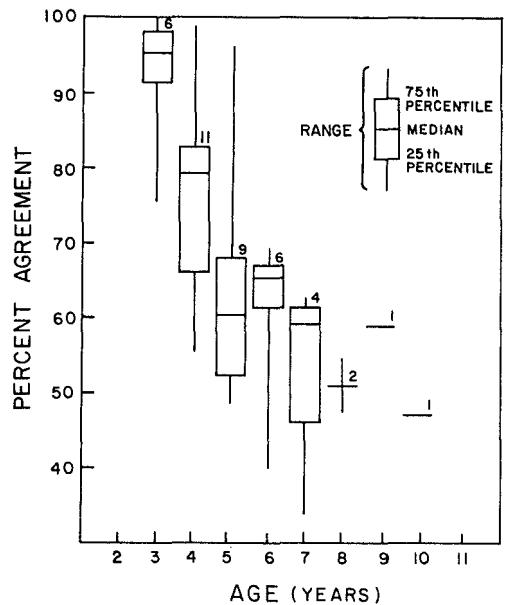


FIGURE 5.— Distribution of percent agreement (number of microgrowth increments divided by number of days between annual measurements (Richardson et al. 1979)) for each age of lot XI hard clams, *Mercenaria mercenaria*. Data from annual shell increments deposited from 1969 to 1971. Number of annual shell increments analyzed at each age is shown.

annually induced MDM in 1969-71 in lot XI hard clams (ages 3-10) are shown in Figure 6. Results from other experimental hard clams were similar. Since microgrowth increments were formed daily, these data indicate that growth rates tended to be slower in summer than in spring or fall of the same year. Median average summer growth rates (dark band) ranged between 21 and 33 $\mu\text{m}/\text{d}$, while those in spring and fall (light bands) ranged between 31 and 48 $\mu\text{m}/\text{d}$, respectively between 1969 and 1971 (Fig. 6). However, these figures represent only growth rates for days of growth and activity; there was a considerable number of inactive days in each annual shell increment (Fig. 5) which would make the actual seasonal average daily growth rate lower. There was also a large range in average increment width in any single band, and individuals in certain annual increments had average increment widths associated with dark bands which were greater than with either or both light bands. This occurred in only 17 of 181 bands analyzed in hard clams from both lots XI and II, or with a frequency of 9%.

Decreased growth rates associated with dark bands were probably due to summer water temperatures above the optimum for growth of hard clams (15°-25°C; Ansell 1968). York River water temperatures

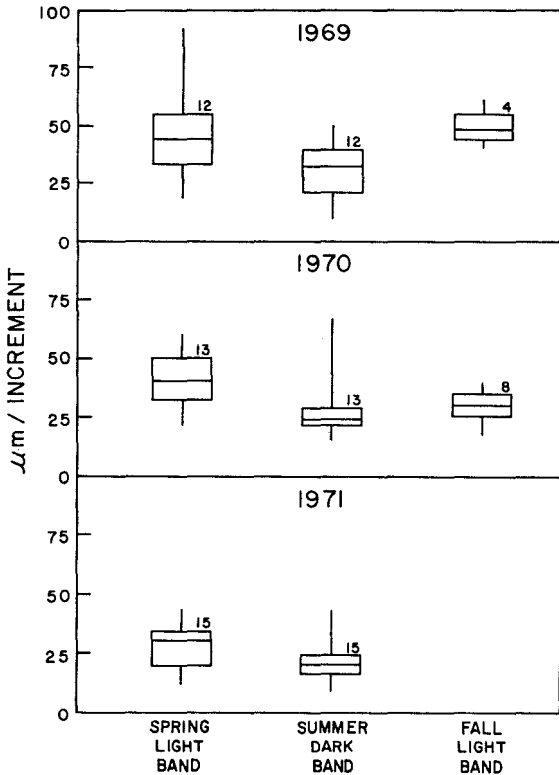


FIGURE 6.—Distribution of average microgrowth increment width ($\mu\text{m}/\text{inc}$) in light and dark bands formed from 1969 to 1971 by lot XI hard clams, *Mercenaria mercenaria*. Figures are drawn as in Figure 5. Increments were counted only between annual measurement disturbance marks in the number of hard clams listed with each spring light or summer dark band. The number of these which had formed light bands prior to measurement each fall in 1969 and 1970 are also shown.

(at 1 m depth) near the location of T and TI hard clams remained above 25°C from 22 June to 26 September 1980, which was approximately the period of dark band formation by all short-term experimental hard clams. For instance, microgrowth increment counts in hard clams collected on 8 August and 1 November 1980 (Fig. 2A, B) date the time of dark band initiation and completion as 29 June and 26 September 1980, respectively.

DISCUSSION

Dark bands in the middle homogenous layer of polished sections and on acetate peels of radial sections of *Mercenaria mercenaria* shells were formed each summer and early fall and were associated with slower growth rates (narrower microgrowth increments) than light bands. Consequently, hard clams from Chesapeake Bay may be aged on the basis

of dark band counts in shell microstructure. Distinct winter growth cessation marks were not formed each year by each individual primarily because of a lack of light band formation in fall, especially by hard clams older than 8 yr. However, hard clams younger than 8 yr also did not form light bands consistently in fall which would separate winter growth cessation marks from summer dark bands. Consequently, dark bands were the only seasonal growth pattern in microstructure which was formed annually by each hard clam analyzed.

The relationships between bands in polished shell section and middle homogenous layer ultrastructure are unknown. According to a theory of growth line formation proposed by Lutz and Rhoads (1977), the ratio of organic matrix to shell carbonates could increase during extended periods of slow shell growth due to dissolution of carbonates in anaerobic (inactive) periods. This may cause shell deposited in summer to appear dark because of higher proportions of organic matrix. However, differences in average microgrowth increment width between light and dark bands in this study were only between 10 and $20\ \mu\text{m}$, which may be too small to cause such fundamental changes in shell appearance. Alternatively, differences in crystal size and/or orientation may also account for bands in the middle homogenous layer. Clark⁷ hypothesized that translucent zones in thin section may result from slightly larger, and more uniformly oriented, crystals. This may result in light transmittance by translucent zones in thin section, and absorption by dark bands in polished shell section. However, it is not known why these areas also appear dark on acetate peels.

Latitudinal variation in seasonal microstructure of *Mercenaria mercenaria* shells is apparent from other studies (Pannella and MacClintock 1968; Rhoads and Pannella 1970; Greene 1975; Kennish and Olsson 1975; Clark 1979). North of Chesapeake Bay (Massachusetts, Connecticut, New York, and New Jersey), distinct winter growth cessation marks were formed each year and the fastest growth during the year occurred most often in summer (Pannella and MacClintock 1968; Rhoads and Pannella 1970; Greene 1975; Kennish and Olsson 1975). However, there was no discussion of association of wide or narrow microgrowth increments with bands in the middle homogenous layer. Seasonal shell microstructure of *M. mercenaria* in Georgia is very similar to that in lower Chesapeake Bay (Clark 1979). Translucent zones, or dark bands, were formed each summer

⁷G. R. Clark II, Department of Geology, Kansas State University, Manhattan, KS 66506, pers. commun. March 1982.

and early fall and were associated with narrower microgrowth increments than opaque zones, or light bands. *Mercenaria mercenaria* in Georgia, however, apparently grow throughout winter since no distinct winter growth cessation marks were observed (Clark 1979). Consequently, two aspects of seasonal shell microstructure appear to vary with latitude: 1) Formation of dark bands or translucent zones in summer and early fall is more common at lower latitudes, and 2) formation of distinct winter growth cessation marks is more common at higher latitudes. These trends are similar, at least in concept, to changes in sublayer crystal structure in the inner shell layer of *Geukensia demissa* which have been observed with latitude (Lutz 1977; Lutz and Rhoads 1978 (see footnote 5); Lutz and Castagna 1980). Latitudinal variation in the ultra- or microstructure of annual shell increments may preclude application of defined increments to all populations along its range.

Latitudinal variation in seasonal water temperature range may be the most important factor regulating seasonal microstructural growth patterns in *M. mercenaria* (Rhoads and Pannella 1970). In this study, it was found that dark band formation tended to occur when water temperatures exceeded 25°C, or the upper limit of the optimum range for shell growth (Ansell 1968). There is little evidence to support the contention that the optimum temperature range, 15°-25°C, changes with latitude in populations of *M. mercenaria* (Ansell 1968). Consequently, growth patterns within shell microstructure may reflect ambient seasonal cycles of water temperature (Lutz and Rhoads 1980).

The relationship between decreased microgrowth increment width (growth rate) as well as location of growth cessation marks with respect to elevated water temperatures has been well documented (Kennish and Olsson 1975; Kennish 1977). Furthermore, circadian formation of microgrowth increments by *M. mercenaria* has also been reported (Pannella and MacClintock 1968; Thompson 1975). However, Pannella and MacClintock (1968), Kennish and Olsson (1975), and Kennish (1980) stated that one increment was formed during each solar day regardless of season or age (up to 8 yr). Each annual shell increment would thus contain about 365 microgrowth increments, and age estimates (in years) could be obtained by dividing counts of all microgrowth increments formed by 365 (Kennish 1980). The results of this study, and that of Crabtree et al. (1979/1980) on daily increment formation by *Chione fluctifraga*, shed doubt on this method of age determination, since the percent agreement between increments and days in annual shell increments

decreased with increasing age. Thus, dividing total microgrowth increment counts by 365 could underestimate age in years.

Decreasing number of days of growth each year with age, as well as individual variability in the number of days of growth in each age group, must be accounted for when shell microstructure of bivalves is used to monitor environmental change. Studies by Kennish and Olsson (1975), Pannella (1976), Kennish (1977), and Jones (1980) are testimony to the quality of information on environmental change stored in bivalve shell microstructure. However, individual variability among bivalves of the same age may require the use of large sample sizes to safely conclude that patterns observed in microstructure of recent or fossil shells were due to changes in environment and not artifacts of individual differences in shell growth.

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