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AGE AND GROWTH, REPRODUCTIVE CYCLE, AND HISTOCHEMICAL TESTS FOR HEAVY METALS IN HARD CLAMS, *MERCENARIA MERCENARIA*, FROM RARITAN BAY, 1974-75.

Raritan Bay has historically supported an abundant hard clam, *Mercenaria mercenaria* L., resource. It was considered the most important commercial species in the bay with an estimated total value of 34 million dollars in 1963 (Jacobsen and Gharrett 1967). Campbell (1967) reported a total standing crop of 4.8 million bushels of clams for the Bay for the same year (3.4 million bushels in New York waters and 1.4 million bushels off New Jersey). More recent estimates are unavailable.

Raritan Bay waters have historically received various domestic and industrial wastes, some of which have had adverse effects on its shellfish resources and fisheries. Raritan Bay was closed to harvesting of hard clams on 1 May 1961, after an epidemic of human infectious hepatitis was traced to the consumption of raw clams from the bay (Campbell 1967). The closure remains in effect to the present time. Zoellner (1977) reviewed the nationwide water quality problems related to shellfish and included Raritan Bay as one of the case studies in the report.

Bivalves accumulate various biological and chemical contaminants by mechanisms related to their filter-feeding habits and transport across their mucous-covered, semipermeable soft body tissues (Goldberg 1957; George 1982). The accumulation of heavy metals, pesticides, polychlorinated biphenyls (PCB's), oil and dispersants, disease causing bacteria, viruses, fungi, parasites, and toxic phytoplankton have serious public health implications and may also adversely affect bivalve resources. Zoellner (1977) has reviewed natural and manmade conditions affecting bivalve populations, including specific studies of the effects of heavy metals, pesticides, and PCB's. McCormick et al. (1984) reviewed physical and sediment characteristics of Raritan Bay, studies of benthic organisms, plankton, and fish, and impact of pollution from sewage, organic chemicals, and heavy metals.

The present study was undertaken to assess potential impacts of contaminants in Raritan Bay on the spawning potential of hard clams. Monthly samples were collected from three study areas within the bay to obtain measurements of the shells, soft body tissues for observations of general condition, and gonadal tissues for observations of the reproductive cycle. Selected specimens were chosen to determine age and growth, and special tissue samples were collected for histochemical tests of certain metals. Published hydrographic conditions and assessment of pollutants in Raritan Bay are discussed in relation to sample results.

Methods

Campbell (1967) described the distribution of hard clams in Raritan Bay and, based on his findings, sites were chosen for repeated collections. The sites were Ward Point, New Dorp Beach, and Horseshoe Cove (Fig. 1). Each was sampled at about monthly intervals beginning on 21 February 1974 and ending on 7 April 1975. The clams were collected by towing a drag-type, nonhydraulic dredge with a 12-in (30 cm) wide knife from the U.S. National Marine Fisheries Service (NMFS) RV *Rorqual*. Tows were made at each site until 30 or more clams larger than 50 mm in shell length were caught. Special collections were made at Ward Point and New Dorp Beach on 1 November 1978 to obtain tissues for histochem-

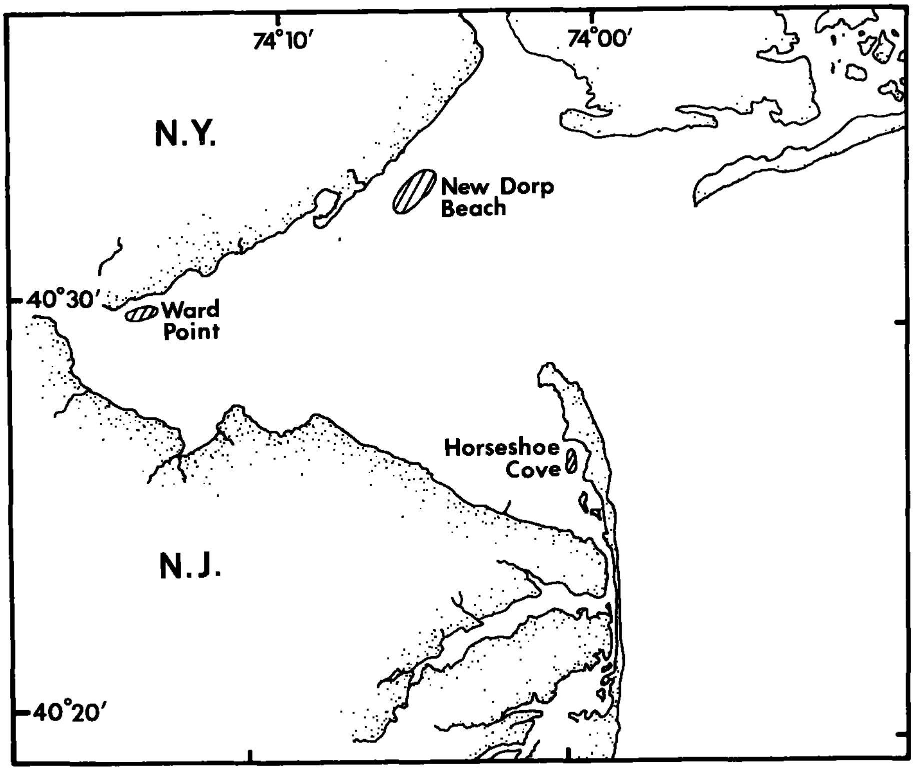

FIGURE 1.—Locations sampled for hard clams, *Mercenaria mercenaria*, in Raritan Bay during 1974 to early 1975 and 1978.

ical tests of some metals, using the same dredge operated from the NMFS RV *Kyma*.

Following sampling operations at each site, the length (longest anterio-posterior dimension), height (deepest dorsoventral dimension from the umbo to ventral shell margin) and width (thickest lateral dimension) of each clam shell was measured to the nearest millimeter with vernier calipers. About 20 clams were then opened and the meat and shell liquor packaged for immediate freezing. Later measurements were made of the drained meat weight, dry meat weight, and percent solids by methods outlined by Ropes (1971a) for other bivalves.

Specimen shells for age and growth observations of hard clams were chosen from Ward Point and New Dorp Beach samples, since clams from these sites exhibited extremes in shell and weight measurements. The selection included five clams in a sample having the smallest mean size, five in a sample having the largest mean size, and those in a sample having a mean size nearly equal to the grand mean for all samples taken at the particular site. The shells were radially sectioned and the cut edge polished to a high luster, as described by Peterson et al. (1983) to facilitate detection of annual growth lines.

Ten additional clams were opened and the soft body tissues were removed for preservation in Bouin's fixative. Methods used for dehydrating, embedding, sectioning, and staining gonadal tissues to prepare slides for microscopic examination of the reproductive cycle were as outlined by Ropes and Stickney (1965) and Ropes (1968). Stages in the development of gonadal tissues were established. The progressive development of sex cells through early to ripe condition and eventual expulsion by spawning activity was a basis for evaluating reproductive viability. Failure to complete all stages of a cycle was considered an indication that the clams were being impacted by environmental conditions.

Specimens were collected for histochemical tests on 1 November 1978 (10 hard clams and 2 oysters from Ward Point and 10 hard clams from New Dorp Beach). The soft body mass of each specimen was removed on the vessel within an hour of capture. One cm-thick slices were dissected from the central body mass of each specimen and immersed in a vial of fixative appropriate for the histochemical test. Samples were then transported to the NMFS laboratory at Oxford, MD, where slides were prepared for microscopic examination. The following histochemical tests were performed:

1. Inorganic iron. Perl's (1867) reaction, reported in Casselman (1959), to produce ferric ferrocyanide from ferric ions (Fe^{+++}).
2. Arsenites and arsenates. The method of Castel (1936), reported in Pearse (1972), to precipitate the cupric salts of arsenites and arsenates (As^{+++}, As^{+++++}).
3. Lead. The method of Cretin (1929), called the chromate method from a reaction with neutral potasssium dichromate, was used, together with the rhodisonate method (Pearse 1972). Both methods are reported in the latter paper.
4. Copper. A method is reported by Uzman (1956) to localize copper by direct treatment of the tissues with rubianic acid. After examining the results of this treatment, the intensification technique to release copper "bound" proteins suggested by Uzman (1956) was also tried.

Results

Hard clams were generally more easily collected at the New Dorp Beach and Horseshoe Cove sites than at Ward Point. Bottom substrata in the dredge with the clams was predominantly a black silty-sand at New Dorp Beach and a relatively clean sand with some shell at Horseshore Cove, but at Ward Point, shell (mostly from oysters) was a major component of the black mud substrata. Live oysters were taken only at Ward Point and their soft body tissues were decidedly green.

Shell and Weight Measurements

Table 1 summarizes the mean, standard deviation, and number of clams for all measurements of clam shell dimensions, weights, and percent solids taken during the study. Values for Ward Point were consistently lower than those for Horseshoe Cove or New Dorp Beach where the values were similar to one another. Student's "*t*" test revealed no significant difference in measurements between Horseshoe Cove and New Dorp Beach ($P > 0.05$), but a significant difference in all measured parameters was found between clams sampled in these areas and at Ward Point ($P < 0.01$) (Table 2).

Age Observations

For Ward Point, age determinations were made for the 5 smallest (collected 13 January 1975), the 5 largest specimens (collected 19 June 1974), and 8 specimens (collected 24 April 1974) which approximated the overall mean shell length. For New Dorp Beach, similar determinations were made for the 5 smallest (collected 21 February 1974), the 5 largest (collected 22 May 1974), and 13 specimens (collected 1 October 1974) which approximated the overall mean shell length. Some shells in the 24 April 1974 Ward Point and 1 October 1974 New Dorp Beach samples were not prepared for age determination, because severe shell erosion at the umbonal area indicated that early age lines would be incomplete.

Two differences were apparent. Firstly, none of the clams from Ward Point was older than 14 years, whereas clams at New Dorp Beach were as old as 20 years. Secondly, clams at Ward Point on

TABLE 1.—Measurements of the shell and meats of Raritan Bay hard clams, *Mercenaria mercenaria*, collected in 1974 and 1975.

	Sample locations								
	Ward Point			New Dorp Beach			Horseshoe Cove		
Measurements	Mean	SD	No.	Mean	SD	No.	Mean	SD	No.
Shell length (mm)	77.3	9.1	221	88.2	12.7	225	87.0	13.2	230
Shell height (mm)	64.6	9.8	221	74.5	11.0	225	72.6	12.7	230
Shell width (mm)	44.8	5.4	221	50.3	7.5	225	50.6	10.1	230
Shell dry wt (g)	95.0	29.5	201	136.2	48.9	225	138.1	48.7	230
Underwater wt (g)	51.4	15.0	221	74.6	26.7	225	77.6	31.3	230
Drained meat wt (g)	1.3	14.1	201	56.9	18.1	205	55.0	20.9	210
Dry meat wt (g)	5.9	2.1	201	9.0	3.5	205	9.1	4.0	210
Percent solids	14.3	3.2	201	15.8	3.7	205	16.5	3.9	210

TABLE 2.—Results of "*t*" *test comparing measurements of Raritan Bay hard clams, Mercenaria mercenaria*, collected in 1974 and 1975.

	Sample locations compared		
Measurements	Horseshoe Cove and Ward Point	New Dorp Beach and Ward Point	Horseshoe Cove New Dorp Beach
Shell length (mm)	9.0313**	10.3795**	0.9857
Shell height (mm)	7.4520**	10.0069**	1.7005
Shell width (mm)	7.5447**	8.8542**	0.3583
Shell dry wt (g)	10.8928**	10.3523**	0.4143
Underwater wt (g)	11.2380**	11.2120**	1.1330
Drained meat wt (g)	7.7373**	9.6513**	0.9866
Dry meat wt (g)	10.0644**	10.7691**	0.2701
Percent solids	6.2215**	4.3548**	1.8704

** = Highly significant ($P < 0.01$).

average were smaller at each age than those at New Dorp Beach (Fig. 2).

Reproductive Cycle

This part of the study focused on a microscopic examination of the cellular events leading up to an accumulation of sex cells in the gonads and their disappearance during the spawning act. Periodic sampling identified the time and duration of spawning. Cellular structures within the sex cells and alveoli walls were important in determining five stages of gonadal condition, as follows:

Females

Early stage. Alveoli semicontracted, basement membrane thickened, and small oocytes embedded in the alveoli walls.

Late stage. Oocytes more numerous at the basement membrane, some of larger diameter extended into the lumina of alveoli, but attached to

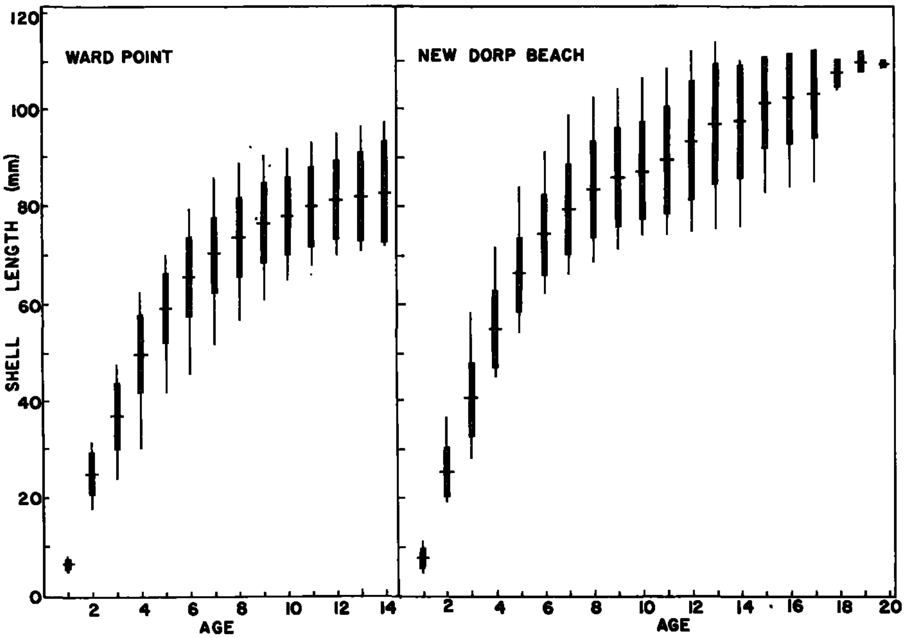

FIGURE 2.—Mean, standard deviation, and range of shell lengths (mm) vs. age (yr) relationships of hard clams, *Mercenaria mercenaria*, at Ward Point and New Dorp Beach, Raritan Bay, 1974-75.

the thickened basement membrane by a stalk. A few of the largest oocytes appear free in the lumina of alveoli. Amphinucleoli prominent in the largest oocytes.

Ripe stage. Numerous large oocytes in the lumina of alveoli. Basement membrane thin, containing only a few developing oocytes. Most oocytes appear free in the lumina of alveoli.

Partially spent stage. Some small oocytes embedded in the basement membrane, with reduced numbers of large oocytes in the alveoli lumina.

Spent stage. A very few of the largest oocytes in some of the alveoli lumina. Basement membrane somewhat thickened and containing small embedded oocytes.

Males

Early stage. Alveoli semicontracted, basement membrane thickened, and follicle cells prominent in the lumina. A few spermatogonia or spermatocytes occur at the periphery of the lumina in most clams, but some with a few spermatozoa scattered in the lumina of some alveoli.

Late stage. Alveoli expanded, basement membrane thin and attached follicular cells less apparent. Primary spermatocytes numerous at the basement membrane, especially during the earliest part of this stage. Secondary spermatocytes and spermatids proliferating into the centers of the lumina. Differentiated spermatozoa arranged in dense radiating bands at the centers of the lumina.

Ripe stage. Lumina of alveoli densely packed with spermatozoa. Fewer spermatocytes and spermatids occurring than in the preceding stage.

Partially spent stage. Few to no spermatocytes or spermatids at the basement membrane. Relatively reduced numbers of spermatozoa in the lumina of alveoli compared to the ripe stage.

Spent stage. Alveoli nearly empty of spermatozoa, but a few near the basement membrane and in the lumina.

For all three sites, gametogenesis progressed from the early to late stages from 21 February to 24 April 1974 (Table 3). Ripening was earliest at Horseshoe Cove, with 10% of the clams in this condition by 22 May and 70% by 19 June. At this latter date, 50% of the Ward Point clams were ripe. Clams at all the sites had ripened by 23 July, and some (20%) at Ward Point and many (70%) at New Dorp Beach were in the partially spent condition, an indication that spawning had begun at these two sites. Spawning was later at Horseshoe Cove, with 67% in the partially spent condition by 21 August. Ripe clams were observed in the sam-

TABLE 3.—Percent occurrence of developmental stages during the reproductive cycle of Raritan Bay hard clams, *Mercenaria mercenaria*, collected in 1974 and 1975.

Sample site and gonad condition	1974									1975		
	2/21	3/28	4/24	5/22	6/19	7/23	8/21	10/1	11/5	1/13	2/6	4/7
Horseshoe Cove												
early	60	70	30	40	10						40	100
late	40	30	70	50	20							
ripe				10	70	100	33	40	30			
part. spent							67	20	60	60		
spent								40	10	40	60	
New Dorp Beach												
early	70	70	70	50							80	90
late	40	20	30	50	100							
ripe						30	30					
part. spent						70	70	50	90	20		
spent		10						50	10	80	20	10
Ward point												
early	40	40	50	40							60	50
late	50	40	50	60	50							20
ripe					50	80	30	30				
part. spent						20	30	50	100			
spent	10	20					40	20		100	40	30

ples from Horseshoe Cove as late as 5 November, but only until 1 October and 21 August at Ward Point and New Dorp Beach, respectively. Partially spent clams were collected from Horseshoe Cove and New Dorp Beach as late as 13 January 1975, but only until 5 November 1974 at Ward Point. The spent and early gametogenic stages identified in clams from all sites by 6 February 1975 indicated that the 1974 reproductive cycle had been completed by all clams and that a new cycle had begun for some. These observations suggest that the spawning period was shortest at Ward Point (5 months) and longest at New Dorp Beach (7 months), with Horseshoe Cove intermediate (6 months).

At all three sites, gametogenesis progressed through morphologically normal stages resulting in the complete spawning of most ripe gametes. Cytolysis of unspent cells was not observed. No hermaphrodites were seen in any of the samples.

The sex ratios of hard clams in the samples were as follows: at Ward Point, 59 males and 60 females; at New Dorp Beach, 56 males and 64 females; and at Horseshoe Cove, 74 males and 45 females. The hypothesis of 1:1 sex ratio was tested by chi-square for all three populations; results indicated a significant ($P < 0.01$) deviation for Horseshoe Cove.

Histochemical Tests for Metals

Histochemical tests were performed on four male and six female hard clams and one male and one female oysters collected at Ward Point; and three male and seven female hard clams collected at New Dorp Beach. The expected histochemical reactions for metals in clam tissues were not observed, i.e., deep Prussian blue for inorganic iron, green granular precipitate for arsenites and arsenates, yellow opaque crystals or scarlet red precipitate by the chromate or rhodizonate methods, respectively for lead, and deep greenish-black precipitate for copper. Thus, the tests for metals in hard clam tissues proved to be negative. Female gonadal tissues tested for arsenites and arsenates from both collecting sites had 1-2 μm diameter granules in the oocyte cytoplasm but the color could not be determined. No similar granules were seen in male tissues. For the two oysters from Ward Point, the deep greenish-black precipitate for copper was evident in connective tissue cells beneath the body wall and palps (a similar reaction was also seen in the epidermal cells of the palps), around the digestive diverticula (Fig. 3), and near the base of cells lining the gills and gut. The connective tissue cells surrounding the male oyster gonadal tubules also tested positive. No similar reaction was seen in the connective tissue cells surrounding the female oyster gonadal tubules, which contained large and apparently normal oocytes. Although not seen in New Dorp Beach hard clam tissues, some gill tissues of hard clams from Ward Point tested for copper showed a slight darkening, but a precipitate was not clearly evident, such as was seen in oysters. The darkening was also absent in underlying connective tissue cells and other tissues. Modification of the technique to intensify the copper reaction was negative for all hard clam and oyster tissues from the two collection sites.

Discussion

Shell dimensions and shell and body weights clearly indicated a smaller size for hard clams at Ward Point than at New Dorp Beach and Horseshoe Cove, which was reflected in the age estimates. Clams at Ward Point were younger (none >14 years) and smaller ($\overline{X} = 77$ mm; none >97 mm) than clams at New Dorp Beach (none >20 years; $\overline{X} = 88$ mm; none >113 mm). These are values much lower than the 111 mm mean and maximum length of 144 mm reported for Nantucket Sound hard clams by Ropes and Martin (1960) and recent, almost 60-yr longevity estimate for the species (Ropes pers. obs.).

Determination of the percentage solids for the meats of bivalves is a measure of condition (Engle and Chapman 1953). The following mean values have been reported: 18.4% for soft-shell clams, *Mya arenaria* (Harriman 1954), 17.0% for oysters, *C. virginica* (Engle 1958), 21.4% for surf clams, *Spisula solidissima* (Barker and Merrill 1967), and 18.5% for ocean quahogs, *Arctica islandica*, (Ropes 1971a). These compare favorably with 16.5% and 15.8% for Horseshoe Cove and New Dorp Beach hard clams, respectively (Table 1). The low value of 14.3% for Ward Point hard clams is an indication of poor condition.

Reported age and growth determinations for hard clams suggest that the Ward Point portion of the Raritan Bay population was being adversely affected. Ansell (1968) has extensively reviewed the literature on annual and seasonal growth of hard clams from various investigations in Canada, the United States, and Europe. Length-on-age observations of the growth of hard clams at sites in Florida, North Carolina, New Jersey,

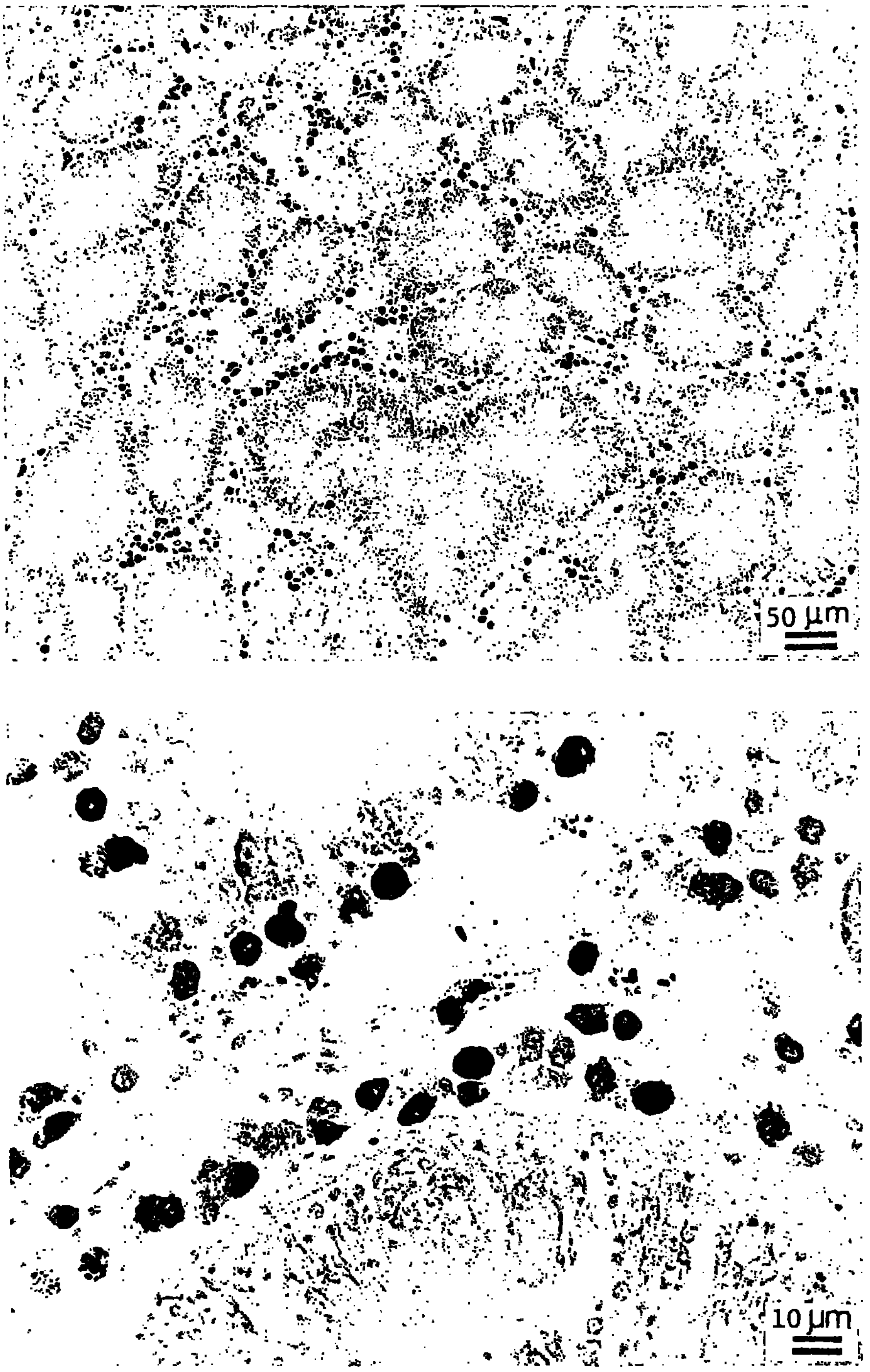

FIGURE 3.—Digestive diverticula of a copper "sick" oyster, *Crassostrea virginica*, from Raritan Bay. Connective tissue cells surrounding the diverticula were greenish black, a positive reaction of copper by the rubianic acid method. A scale of magnification appears in the lower right-hand corner of each photomicrograph.

New York, Massachusetts, Maine, and Canada were compared with the data for the hard clams sampled at the New Dorp Beach and Ward Point, Raritan Bay sites. Growth of Raritan Bay clams was about midway between the fastest (Florida) and the slowest (Canada). At Ward Point, growth to age 4 was about equal to New Jersey growth, but at New Dorp Beach, growth was consistently greater. After age 4, clams at Ward Point grew much slower than New Jersey clams, and even slower than Maine clams after age 5. These observations support the conclusion by Ansell (1968) of extreme local variations in the annual growth of hard clams.

The absence of large, old hard clams in the present samples may be the result of pollution effects, as Jefferies (1972) found for Providence River, RI, hard clams stressed by hydrocarbons. A high C_{15+} hydrocarbon value of 3,672 ppm in sediments at Ward Point was reported by Koons and Thomas (1979). Nevertheless, mortalities (e.g., paired valves containing dead bodies) were not evident at any site.

Food availability is considered an important factor for growth of hard clams by Ansell (1968). Jefferies (1962) considered the nutrient content of Raritan Bay to be rich and the environment capable of supporting dense biotic communities, because of a sluggish circulation pattern. Patten (1962) found that phytoplankton species diversity decreased up bay (towards Ward Point) from the higher values in the Lower Bay. A lower diversity of phytoplankton in the vicinity of Ward Point may have resulted in lower amounts of food organisms being available for the nutritional needs of the clams, and was probably reflected in their slower growth and poor meat condition.

Gonadal development culminated in spawning at three Raritan Bay sample sites. This suggests that the reproductive capacity of hard clams in Raritan Bay was not being affected by pollutants. However, some differences were noted in a comparison of the results based on available information about the time and duration of spawning and larval production at several northwestern Atlantic coast locations. At more northern locations, Belding (1912) and Deevey (1948) in Massachusetts, Landers (1954) in Rhode Island, and Carriker (1959, 1961) in Long Island, NY, and Little Egg Harbor, NJ, observed that spawning was initiated 1 to 2 months earlier than was observed in Raritan Bay during 1974. Similarly, at more southern locations, Keck et al. (1975) in Delaware, Sieling (1956) in Maryland, Ropes (1971b) and Chanley and Andrews (1971) in Virginia, Porter (1967) in North Carolina, and Eversole and Michner (1980) in South Carolina observed that spawning was initiated 1 to almost 3 months earlier. A spawning beginning about three-fourths of a month earlier than the present study was observed by Jefferies (1962) in Raritan Bay, but Loosanoff (1937) in Long Island, NY, observed that spawning began at the same time as in Raritan Bay during 1974. No particular trend in the time of peak spawning was evident in the several studies, except that the peak spawning in 1974 at all Raritan Bay sample sites was later than reported in any of the other studies. Spawning ceased somewhat earlier at more northern locations, and was not as prolonged at more southern locations as was observed in Raritan Bay during 1974.

The project was initiated under the premise that heavy metal pollution in Raritan Bay could be affecting the viability of adult hard clams. Studies indicated that tests for copper and lead should be specifically included, because high concentrations of both have been found in Raritan Bay sediment and water samples (Greig and McGrath 1977; Waldhauer et al. 1978).

The negative results of histochemical tests for heavy metals in Raritan Bay hard clams are not readily explained. Eisler (1981) has listed studies that found 15 heavy metals (including those tested for in the present study) in field collected hard clams. However, heavy metals can occur in several forms (Waldichuk 1979; Fayi and George 1985), suggesting that the histochemical tests may not have been specific for those occurring in Raritan Bay hard clams. Pringle et al. (1968) reported lower levels of copper in field collected hard clams than oysters (*Crassostrea virginica* and *C. gigas*). The positive result for copper in the oysters from Ward Point is probably related to the species greater sensitivity and accumulation of more of the metal in their tissues than hard clams. Copper may have been at a level too low for detection by the histochemical test, although limits for detection of copper or other metals were not given in Pearce (1972).

Hydrographic conditions (not specifically sampled for during the present study) probably influenced the growth and survival of hard clams in Raritan Bay. Based on current flow observed by Jefferies (1962) and Patten (1962), the New Dorp Beach area is influenced principally by water from the Hudson River and the ocean; the Ward Point area is influenced by an eddy formed from the westward flow of water from the ocean, and flows from the Arthur Kill and Raritan Rivers; the Horseshoe Cove area is affected most strongly by water flowing from the Shrewsbury River.

Ansell (1968) analyzed data from throughout the geographical range of adult hard clams to develop a relationship between temperature and growth rate. Shell growth occurred between temperatures of 9°-31°C and ceased at lower and higher temperatures; the optimum was 20°C. Castagna and Chanley (1973) reported a salinity tolerance range of 12.5-46‰ for survival of adult hard clams, with an optimum of 24-28‰.

The above temperature and salinity limits for adult hard clams were compared with hydrographic results reported by Jefferies (1962). He listed mean surface and bottom water data beginning in summer 1957 to summer 1958 at two locations (Stations 1 and 6) in Raritan Bay near the Ward Point and New Dorp Beach sample sites, respectively. Throughout Raritan Bay no growth of adult clams would be expected during winter due to low bottom temperatures (2.3°-3.0°C); slow growth would occur during the increasing and decreasing temperatures of the spring and fall. Near normal growth probably occurs at New Dorp Beach and Ward Point during the summer when temperature means appeared to be near optimum conditions. Lowest bottom salinities were recorded during the spring at Jefferies' (1962) station number 1 near Ward Point. These values indicate that the minimum salinity tolerance limit for adult hard clams may occasionally be reached in the area. Salinities near the New Dorp Beach area (Jefferies 1962, station 6) were all within the tolerance limits for adult hard clams.

Jefferies (1962) reported dissolved oxygen measurements and found relatively low concentrations in the water near Ward Point in both summer periods. Slightly higher values occurred in the fall of 1957 and following spring near Ward Point, but the confidence intervals were greater than for any other period, indicating more variable conditions. Dissolved oxygen levels near New Dorp Beach were consistently higher than near Ward Point.

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