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# UNEXPLAINED MORTALITIES OF HATCHERY-REARED, JUVENILE OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN)

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***ABSTRACT*** Survival, growth and pathology of juvenile oysters, *Crassostrea virginica*, in off-bottom culture at Oyster Bay and Fishers Island, New York, were monitored during the summer of 1991 to document and help explain the episodic mass mortalities of cultured seed oysters that have occurred in the northeastern USA over the past several years. At Oyster Bay, where the more detailed study was conducted, 54 to 75% losses affected several 1991 cohorts at mean shell heights ranging from 15 to 24 mm, within 3 to 6½ weeks of transfer from the hatchery to growout trays. Mortalities occurred in July and August, at temperatures between 22 and 25°C, and were reduced significantly at low stocking densities. Deaths were associated with reduced tissue and shell growth, reduced condition index, mantle retraction, the deposition of an abnormal conchiolin layer on the inner shell, and lesions of the mantle surface. No obvious pathogen was identified in soft tissues or shells by light or electron microscopy. The pathology suggested that a toxin-producing agent of bacterial or microalgal origin, or chemical contaminant, caused mantle retraction and secretion of anomalous conchiolin as a defense mechanism. Two potential agents were recognized. Bacteria were found in mantle lesions and within the abnormal conchiolin sheet, but not consistently and with <30% prevalence; it is not clear whether these were primary or secondary invaders. Blooms of a large dinoflagellate, *Gymnodinium sanguineum*, occurred at peak densities of $5 \times 10^5$ cells $l^{-1}$ at the time of initial oyster mortalities, although the species is not known to be toxic to bivalves. Follow up studies are planned to identify the etiological agent and culture methods that minimize losses.

***KEY WORDS:*** *Crassostrea virginica*, juvenile oysters, mortalities

## INTRODUCTION

Heavy, unexplained mortalities of hatchery-reared, juvenile oysters, *Crassostrea virginica*, have been documented since 1988 by commercial growers in the northeastern U.S. (Rask 1990, 1992; Relyea 1992), and constitute one of the major impediments to the expansion of aquaculture oyster production in this region. Losses typically occur during early field growout of off-bottom cultures. Before 1991, oyster mortalities were observed in the Damariscotta River, ME (>90% losses in 1988, and 40 to 90% losses in 1989); at several sites in MA in 1989 [Orleans (>95% losses), Nantucket (80% loss), and Essex]; in Oyster Bay, Long Island, NY (50% mortalities in 1990), and West Harbor, Fishers Island, NY (1988 through 1990) (authors' 1992 survey). No mortality episodes were documented in Maine or Massachusetts in 1990, but these recurred in the Damariscotta River in 1991.

Juvenile oyster mortalities have thus been widespread and recurrent, but may not be related to a common cause. Some common features were described, however, at most of these sites: 1) mortalities generally occurred during the summer (primarily in July–August) after a period of sustained growth; 2) losses were highly age/size specific, preferentially affecting first-year oysters, at a size of about 6 to 30 mm; 3) mortalities occurred in surface trays or suspended culture, not in bottom plantings; 4) affected oysters

exhibited generalized symptoms of stress, including emaciation of tissues, shell deformity (cupped, left valve outgrowing the right valve), and in some cases, fragile, poorly calcified shells, and 5) other bivalve species cultured in the same system were generally unaffected (e.g., hard clams, *Mercenaria mercenaria* in Oyster Bay, NY, and the European oyster, *Ostrea edulis*, in the Damariscotta River, ME). Mortalities could not be traced to a single hatchery or a common broodstock. Tissue samples were collected at several sites and examined by pathologists at several laboratories. Standard techniques for the detection of known oyster pathogens, including tissue section histology, failed to demonstrate any recognized organism (Rask 1990).

Existing documentation of episodic juvenile oyster mortalities was limited or anecdotal, and insufficient to allow more than speculation of associated causes. Mortalities could be attributed to one or a combination of factors, including primary or secondary infection by a undetected pathogen, or environmental stress due to anthropogenic contaminants, toxic and/or nutritionally unsuitable microalgae, hypoxia, extreme temperatures or overcrowding. Increased susceptibility to disease or other stress factors may also be related to the organisms' genetic makeup (e.g. Ford 1988), as well as their prior history and physiological condition during post-settlement stages in the hatchery, prior to transfer to the field.

The goal of the present study was to implement a rapid-response, comprehensive sampling program that would document juvenile oyster mortalities and help to identify potential cause(s) at two non-contiguous growout sites: Oyster Bay, NY (Frank M. Flower and Sons, Inc.) and Fishers Island, NY (The Clam Farm Inc.). Flower & Sons is the leading producer of oysters in New York State and has successfully grown oysters with current technology for over 30 years. The Clam Farm has been growing oysters on a much smaller scale since 1988. Specific objectives of the study were to describe the relationship between oyster growth, mortality and environmental parameters (temperature, salinity and phytoplankton composition), and to characterize histopathology of the juvenile oyster mortality syndrome as well as to identify potential organisms associated with affected oysters at these two sites.

## MATERIALS AND METHODS

### *Sampling Program at the Oyster Bay Study Site*

We monitored growth, mortality, and histopathological condition of two 1991 oyster cohorts produced at the Flower hatchery, which were held in a growout raft system in Mill Neck Creek, Oyster Bay, on the north shore of Long Island, NY. These oysters were the product of two spawnings, conducted on March 18 (large cohort) and April 25 (small cohort) 1991, using local broodstock from Oyster Bay (different individuals for each spawn). Experimental oysters were set on 0.2 to 0.8 mm crushed hard clam shell, and moved from the hatchery to floating trays for field growout on May 25 (large cohort) and June 3 (small cohort). On June 14 mechanical grading of each cohort yielded two experimental groups of relatively uniform size (referred to hereafter as small and large cohorts, SC and LC, respectively), which averaged 6.4 and 16.1 mm in shell height (greatest dimension from the umbo to the posterior margin of the shell) respectively. Oysters were placed in 0.8 m × 1.2 m × 8 cm trays, open at the top and lined on the bottom with 1 mm mesh window screen. Stocking densities included one typically used for commercial growout by the Flower Co. (hereafter referred to as high density) and one lower density (Table 1). The low density treatment was included to determine if growth and mortality in the trays were density-dependent.

Large and small cohorts were suspended in the water column (depth = 3.7 m at low water) in adjacent stacks of six trays each. High-density experimental oysters of a given size were held in the top and bottom trays of each stack (trays 1 and 6, respectively), and low-density groups in trays 2 and 5. The upper tray was suspended about 12 cm below the surface, while the lower tray remained at least 2.5 m off-bottom. Two replicate stacks were maintained for each cohort. During maximum summer production, up to 432 stacks (2592 trays) are typically used to grow oysters and hard clams at this site, with clams occupying only up to 12–23% of available space (J. Zahtila, Flower Co., pers. comm.).

Oysters were sampled (without replacement) approximately every 2 weeks between June 14 and September 20, 1991, although mortalities of small oysters were determined through November 7. A random sample of each replicate was removed after thoroughly mixing the contents of two trays from a given stack (e.g. 1 and 6). Because the growout area is relatively shallow and well mixed, no attempt was made to resolve differences in growth and mortality with depth. Oysters were thinned by random removal of oysters over time (see Table 1). Stocking densities and thinning frequency of the high-density group were decided upon by Flower's personnel. No grading and culling of live oysters, a standard procedure employed by commercial growers, was carried out during the present study. Mechanical grading and culling of dead oysters, however, was conducted on August 9 on a rotary, cylindrical drum sieve. This was necessary because accumulation of shell debris and large numbers of dead oysters, which were by then significantly smaller than live individuals, interfered with effective sampling. Culling removed only dead oysters and did not affect the size distribution of survivors. To allow calculation of cumulative losses, mortality of oysters retained in the system was determined immediately prior to, and following culling. Grading/culling on August 9 effectively removed dead oysters from the small cohort (e.g. 92% of dead oysters from high density trays), but for unknown reasons removed only 34 to 48% of those from the large cohort, and therefore a second culling of this cohort was carried out on September 20 (Fig. 1).

Thus, stocking densities (of the high-density group) and handling protocols were kept similar to standard commercial practices, except that grading and culling was minimal throughout the study, and the identity of experimental oyster groups was maintained over time. Additionally, experimental oysters were held in suspended culture longer than normal at this commercial facility, where oysters are generally removed from growout trays and planted on the bottom at about 20–30 mm in shell height.

On July 26, a third group of oysters, referred to as the "late cohort," was included in the sampling program. These oysters originated from a June 6 spawning of Oyster Bay broodstock (different individuals than those used to produce earlier experimental groups), set on June 27, and were moved to floating trays on July 26, at an initial mean size of 7.7 mm (SE = 0.2, n = 42). This cohort was sampled at weekly intervals, but data are available only through August 29, since the identity of this group was not maintained after this date.

Surface water temperature, determined with a hand-held thermometer, and salinity, determined with a refractometer, were measured at least twice a week at the growout location. Surface water samples were collected weekly and preserved with Lugol's

**TABLE 1.**

**Stocking conditions of oysters cultured off-bottom at the two study sites. Densities are given in numbers of oysters (or volume in liters) per culture unit (tray or pearl net). Number of packed oysters per unit volume was determined in triplicate from subsamples; NT = oysters not thinned at this date.**

A) Oyster Bay study site (date of deployment = June 14, water temperature = 21.5°C); $\overline{H}$ = mean shell height; HD and LD = high and low density experimental groups. Date notation = month/day.

| | Small 1991 Cohort | | Large 1991 Cohort | |
|---|---|---|---|---|
| Initial $\overline{H}$ (mm) | 6.4 | | 16.1 | |
| (SE, n) | (0.12, 50) | | (0.30, 50) | |
| | Stocking densities (#/tray; volume (l)/tray) | | | |
| **Date** | **HD** | **LD** | **HD** | **LD** |
| 6/14 | 35,700 (2.4) | 3,800 (0.25) | 7,200 (6.0) | 2,000 (1.7) |
| 6/28 | 19,680 (6.0) | NT | 3,680 (8.0) | NT |
| 7/11 | 3,360 (4.0) | 2,232 (3.6) | NT | 2,106 (3.6) |
| 7/26 | 2,340 (6.0) | 1,170 (3.0) | 1,540 (8.8) | 700 (4.0) |
| 8/9 | 1,424 (8.0) | 572 (4.0) | 1,167 (9.0) | 465 (4.5) |
| 8/23 | 996 (12.0) | 431 (5.2) | 484 (11.0) | 264 (6.0) |
| 9/6 | 576 (12.0) | 192 (4.0) | 400 (10.0) | 111 (3.0) |
| 9/20 | 370 (10.0) | NT | 392 (8.0) | NT |

B) Fishers Island study site (date of deployment = June 12, water temperature = 18°C).

| | Small 1991 Cohort | Large 1990 Cohort |
|---|---|---|
| Initial $\overline{H}$ (mm) | 8.9 | 31.7 |
| (SE, n) | (0.23, 49) | (0.92, 30) |
| Stocking density (#/pearl net) | 500 | 200[a] |

[a] Thinned to 100 oysters/pearl net from July 25 onward.

iodine solution, in its concentrated acidic version (Throndsen 1978), for determination of phytoplankton species composition and cell concentrations. Population densities of phytoplankters greater than 5 μm were determined using a Sedgwick-Rafter chamber. A numerically dominant algal species, *Gymnodinium sanguineum* (Hirasaka) (=*nelsoni* = *splendens*), was counted at 200× magnification in unconcentrated samples. For other species, cells in water samples were first concentrated by centrifugation, and enumerated at 400×. The 95% confidence interval was estimated according to Venrick (1978).

Qualitative, visual assessments were made on the degree of siltation and fouling of trays, prevalence of pale digestive glands (a gross indicator of feeding inhibition), and mud blisters (presumably caused by the boring polychaete *Polydora* sp.) determined by dissecting 15 oysters from each cohort.

***Determination of Mortality and Growth***

Percent mortality was determined *in situ* from a representative sample of at least 100 oysters from each replicate, by prying open the valves with a scalpel, and determining the presence/absence of tissues attached to the shell. Live oysters were returned to the laboratory, where any additional deaths undetected in the field sampling were determined following dissection for dry weight and condition index determination. Counts of disarticulated cupped (left) valves were included in mortality estimates.

At each sampling date, 22 to 50 live oysters from each of two replicate groups were measured with digital calipers (±0.1 mm) to obtain shell height (H). Tissues were dissected and oven-dried to constant weight at 50°C to determine dry weight, using an analytical balance (±0.1 mg) or Cahn electrobalance (±0.01 mg) as appropriate. The sample size was increased to 50 oysters per replicate from July 26 onwards, to accommodate increasing variability in size over time. Whole body weight of tightly-closed oysters, air-dried at room temperature, and dry weight of shells was also determined to estimate the condition index. Soft tissue weight and condition index of small oysters were not determined on the first sampling date (June 14), because oysters could not be reliably shucked at this small size. The following gravimetric condition index (CI) was determined:

$$CI = [\text{Dry meat weight (g)} \times 1000/\text{Internal shell cavity capacity}],$$

where internal shell cavity capacity (g) = (whole live body weight in air) − (dry shell weight in air), following removal of epibionts and debris from the valves (modified from the formula provided by Lawrence and Scott 1982). The gravimetric CI has been recommended as the standard index of choice to measure the nutritive status and meat yield of oysters (Crosby and Gale 1990), and has been ranked as the most sensitive out of 21 indices commonly employed for oysters (Bodoy et al. 1986). The incidence of abnormal conchiolin deposition on the inner shell surface was recorded from July 11 onwards.

***Histopathology***

Histopathology was performed at the Haskin Shellfish Research Laboratory (HSRL) of Rutgers University, the Battelle Ocean and Marine Sciences Laboratories (Battelle), and the Virginia Institute of Marine Science (VIMS). For light microscopy, a minimum of 25 randomly chosen oysters (including both live and gaping oysters) from each experimental cohort were preserved in

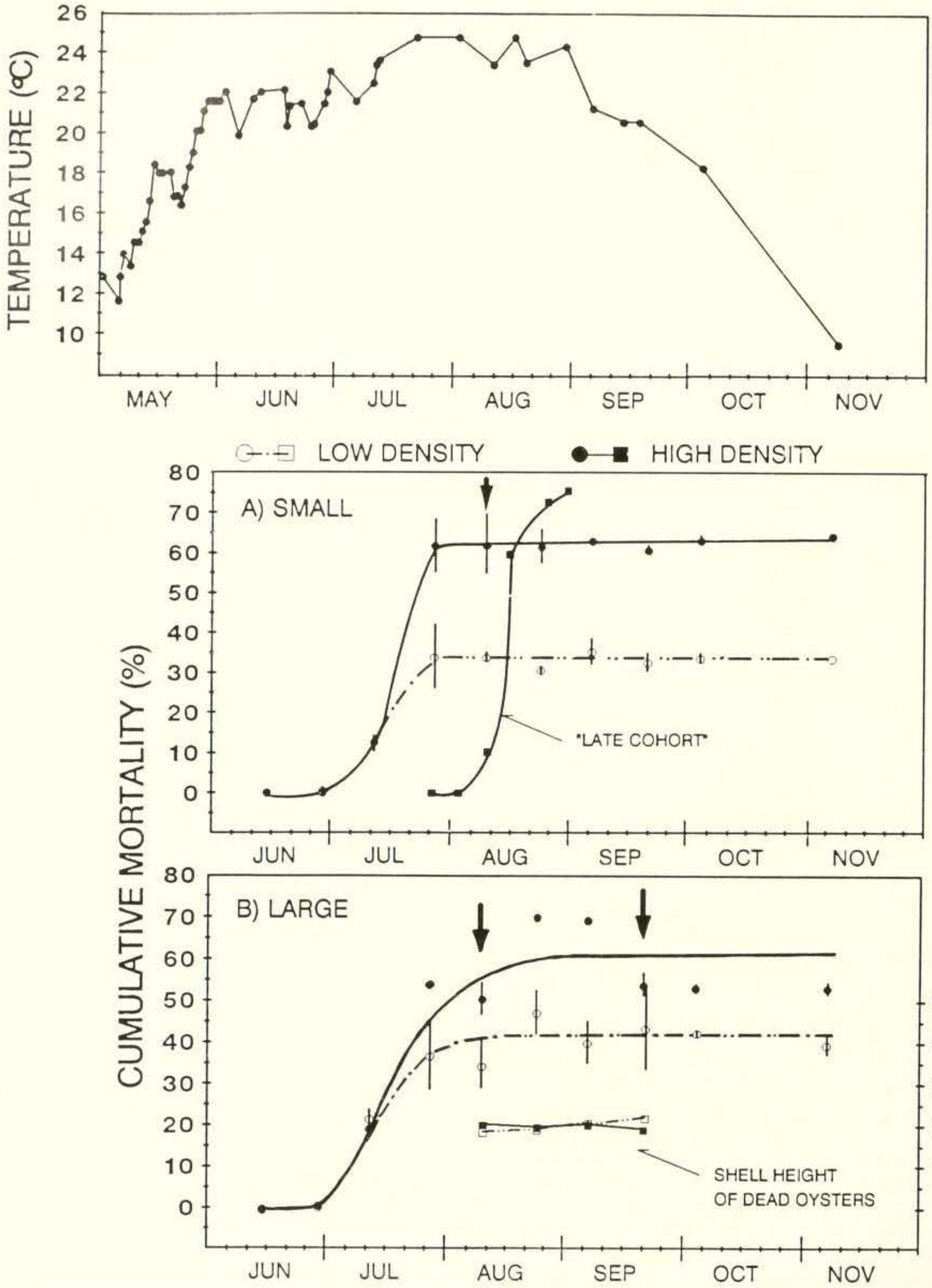



**Figure 1.** Water temperature at the Oyster Bay, NY oyster growout site (top graph), and mean cumulative, percent mortality of experimental cohorts of juvenile oysters, *Crassostrea virginica* (±standard error, SE; n = 2): A) small cohort and "late cohort," B) large cohort (eye-fitted curves; see text and Table 1 for initial cohort age and size characteristics, and stocking densities). Arrows mark the timing of removal of dead oysters from growout trays by mechanical grading. Bottom graph also shows the mean shell height ($\overline{H}$) of dead oysters (n = 51 to 86, and 23 to 70 for high and low density groups respectively), in order to demonstrate that no additional mortalities occurred after the August 9 sampling date (see Fig. 2 for $\overline{H}$ of live oysters at the corresponding sampling times).

Davidson's fixative (Shaw and Battle 1957) immediately upon collection. After 24–48 h, they were transferred to this same fixative, but without acetic acid, for long-term storage. Samples were shipped to the HSRL for histological processing and analysis. Small oysters which could not be readily dissected *in situ* were fixed whole, following careful prying open of their valves. Large oysters were shucked, and their shells were discarded. The shells of small oysters had decalcified by the time the oysters were processed for histology. Samples examined at Battelle were shipped approximately every 2 weeks, from mid-June through mid-November, by overnight mail from the Flower Co. They were then decalcified and fixed in Dietrich's fixative. A number of individuals were examined prior to fixation for evidence of fungal infection or other organisms that might have penetrated the shell.

Fixed tissues of large oysters were cut laterally from the hinge region, through the adductor muscle, to the posterior margin before being embedded in paraffin. Small individuals with decalcified shells were embedded intact with the ventral side down. This orientation allowed us to view the epithelium under the hinge ligament, the myoepithelial attachment of the adductor muscle to the shell, the abnormal conchiolin layer when present, and the periostracum-secreting mantle edge, as well as various internal organs.

Embedded tissues were sectioned serially at 5–6 μm, and stained with hematoxylin and eosin, or a Masson's Trichrome stain (Humason 1979) modified by the addition of Fast Green and Orange G. An initial sample of 55 individuals, collected during peak mortality and categorized as showing a) no sign of distress, b) early distress (some overgrowth of the left valve, some conchiolin deposition of inner valve, or both), or c) advanced distress (clear overgrowth of left valve, heavy abnormal conchiolin, weak muscle attachment), was examined microscopically for histopathological conditions. Pathological conditions recognized in the initial sample (see Results) were identified and rated (none, light, moderate, or heavy) in subsequent bi-weekly samples.

For transmission electron microscopy, mantle tissue and conchiolin were fixed in 2% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M Millonig's phosphate buffer with 2.7% glucose at pH 7.3 for 2 hr followed by buffer rinses in 0.2 M Millonig's phosphate buffer with 5.4% glucose at pH 7.3, then post-fixed in 1% $OsO_4$ with 0.1 M Millonig's and 2.7% glucose at pH 7.3 for 1 h. After washing in distilled water, the tissue and conchiolin blocks were stained in 1% aqueous uranyl acetate for 1 h followed by dehydration in a graded series of ethanol solutions and transfer into propylene oxide. Infiltrations and embeddings were made in Spurr's resin and thin sections were stained in Reynold's lead citrate and uranyl acetate. All procedures were accomplished at room temperature except 1) post-fixation which was done in an ice bath, and 2) resin polymerization which was performed at 58°C.

### *Sampling Program at the Fishers Island Study Site*

A similar sampling protocol was implemented at a more oceanic site on the north shore of Fishers Island, NY: 2 cohorts of cultchless juvenile oysters were deployed in pearl nets (34 × 34 cm basal area), in vertical arrays of 4 nets, on June 12, 1991, in eastern West Harbor (depth = ca. 3 m at low water). Both cohorts were produced at the Aquacultural Research Corporation hatchery, Dennis, MA, using broodstock shipped from Island Pond, Fishers Island (G. Matthiessen, Ocean Pond Corp., pers. comm.). The small (1991) cohort (SC) was grown in trays at Ocean Pond Corp.'s nursery site in Island Pond, prior to transfer to West Harbor in mid-June. The large (1990) cohort (LC) overwintered in pearl nets in Island Pond until June 1991. Large cohort oysters were held in pearl nets with a 6 mm mesh, whereas SC oysters were held in 3 mm pearl nets (see Table 1 for initial sizes and stocking conditions). Low stocking-density groups were also maintained at this site (50 and 100 large and small oysters per pearl net respectively), and sampling was without replacement, as described previously. Live oysters were shipped to SUNY Stony Brook by overnight mail in coolers containing freeze-packs. Experimental oysters were thinned but not graded or culled, except at the time of initial deployment.

## RESULTS

### *Sampling at the Oyster Bay Study Site*

#### Mortality Patterns

Mortality estimates obtained *in situ* were generally in excellent agreement with those determined following dissection of oysters in the laboratory. Greatest discrepancies occurred on July 11, when field-determined mortalities were 4–9% and 9–12% for small and large oysters respectively, while laboratory-derived values were 10–15% and 18–21% respectively.

Despite variability between replicate stacks at any given sampling date, especially for small oysters, there was no consistent trend showing greater losses within one of the two replicates. Thus, these differences are attributed to sampling artifact, and data averaged for the two replicates.

Cumulative mortalities remained negligible until July 11, when they reached 12% and 20% for the small and large cohort, respectively, at a time when the water temperature reached 24°C (Fig. 1). Mortalities peaked at 62% and 54% on July 26 for small and large cohorts, respectively, and ceased thereafter. Grading/culling conducted on August 9 was not 100% effective in removing dead oysters from the LC. Therefore, an apparent increase in mortalities of large oysters between August 9 and August 23 is attributed to sampling error, rather than new mortalities. This was confirmed by determining the mean size of dead oysters over time (Fig. 1B), which remained constant, at 20.3–20.8 mm [the size of oysters in early July (Fig. 2)], between August 9 and September 20. Thus the dead oysters present in late August samples are clearly remnants from the July mortality outbreak.

Losses were consistently lower for oysters stocked at the lower density. They attained maxima of only 30–37% for small oysters and 28–38% for large oysters on July 26, but their timing coincided with that of oysters held at high density. Mortalities of the LC levelled off between 40 and 47% after August 23 (Fig. 1B). The outbreak of mortalities occurred during a period of elevated surface temperatures (range = 21.7 to 25.0°C), which were maintained between June and early September (Fig. 1). A comparison of Flower's temperature records from 1987 through 1991 (May 1 to July 26) indicates that late spring water temperatures were higher than usual in 1991. The mean for May was 17.2°C in 1991, compared to 14.2 and 15.3°C in 1990 and 1989, respectively. Cumulative day-degrees calculated for May were 8 to 16% higher in 1991 than in the 4 previous years. However, heavy oyster mortalities were also experienced in 1990, when the lowest May to July temperatures were recorded. Salinities ranged between 24 and 28 ppt, and only moderate to low levels (in late September and early October) of fouling and siltation of trays were observed throughout the study period.

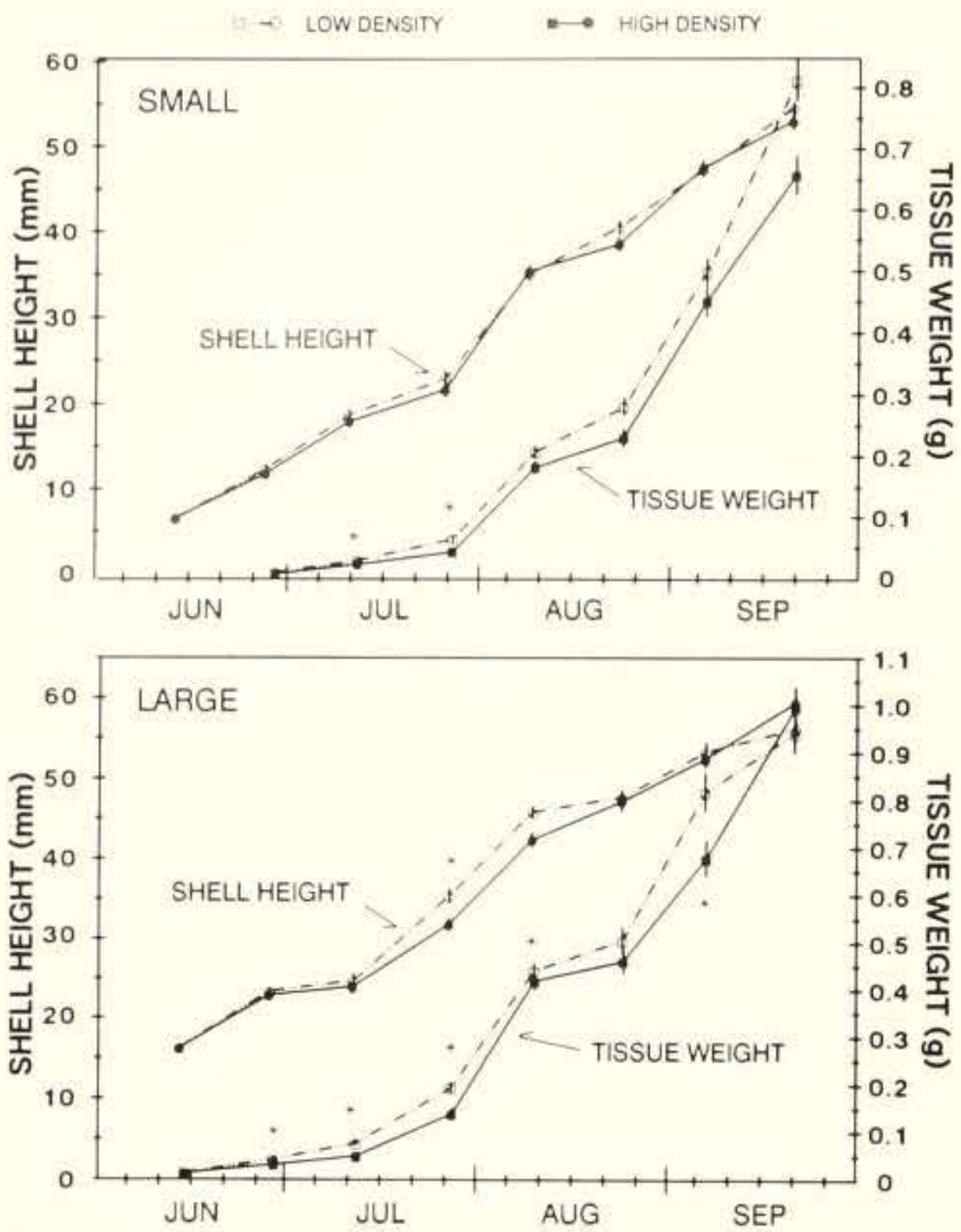



**Figure 2. Temporal changes in mean shell height and dry weight of soft tissues of small and large oyster cohorts held at 2 stocking densities in Oyster Bay, NY. Error bars as in Fig. 1 (pooled data from 2 replicate experimental groups, n for samples collected through July 11 = 46–50; n for remaining samples = 94–100). Asterisks indicate dates when significant ($p < 0.05$) differences were observed between density treatments.**

The "late cohort," which was the last cohort produced at the Flower Co. in 1991, suffered heavy mortalities 2 to 3 weeks later than the main experimental groups. Cumulative losses increased from 7–13% on August 9 to 75% on August 23, a period during which temperatures remained constant at about 23°C.

On July 4, live, gaping and dead oysters from non-experimental groups, which were experiencing heavy mortalities at the growout site, were examined microscopically for the presence of predatory flatworms, *Stylochus* sp. These were not observed in any of the samples, and thus were eliminated as a potential cause of oyster mortalities. These results were independently corroborated by M. Castagna, who examined live oysters at this site on July 9 (VIMS, pers. comm.). Mud blisters were commonly observed in shells of September 20 samples, and were prevalent in October 3 samples from both cohorts, but were not observed on earlier sampling dates.

### Phytoplankton

Analysis of phytoplankton samples revealed that a bloom of the unarmored dinoflagellate *Gymnodinium sanguineum* (cell length 43 to 50 μm, width 31 to 43 μm) occurred in July, at the time when the two main experimental cohorts suffered high mortalities, and again in September. Cell densities of *G. sanguineum* peaked at $5.1 \times 10^5$ cells $l^{-1}$ on July 20, declined rapidly by July 26 and remained at relatively high levels, 5.0 to $7.1 \times 10^4$ cells $l^{-1}$, throughout early August (Fig. 3). Cell densities of this species peaked again at $3.5 \times 10^5$ cells $l^{-1}$ on September 20, when the small and large cohorts had attained 52.9 and 58.4 mm in shell height respectively. The dominant diatom species was *Skeletonema costatum*, and flagellates were mostly composed of cryptomonads (Fig. 3). During the bloom period, *G. sanguineum* was not necessarily the numerically dominant phytoplankton species. However, if we consider the size difference between a *G. sanguineum* cell and a *S. costatum* cell (5 to 8 μm), the former species was definitively the major contributor of phytoplankton biomass. Starting on July 20, a certain percentage of *G. sanguineum* cells observed were partially degraded or covered by fungus-like hairs. The cell counts reported were determined from surface water samples, and may not be representative of the entire water column, since *Gymnodinium* spp. are motile and positively phototactic, and may not be homogeneously distributed in depth even in shallow estuaries (Fiedler 1982, Chang and Carpenter 1985).

### Growth Patterns

Differences in mean height or soft tissue weight between replicates at each sampling date were tested using paired t-tests, ad-

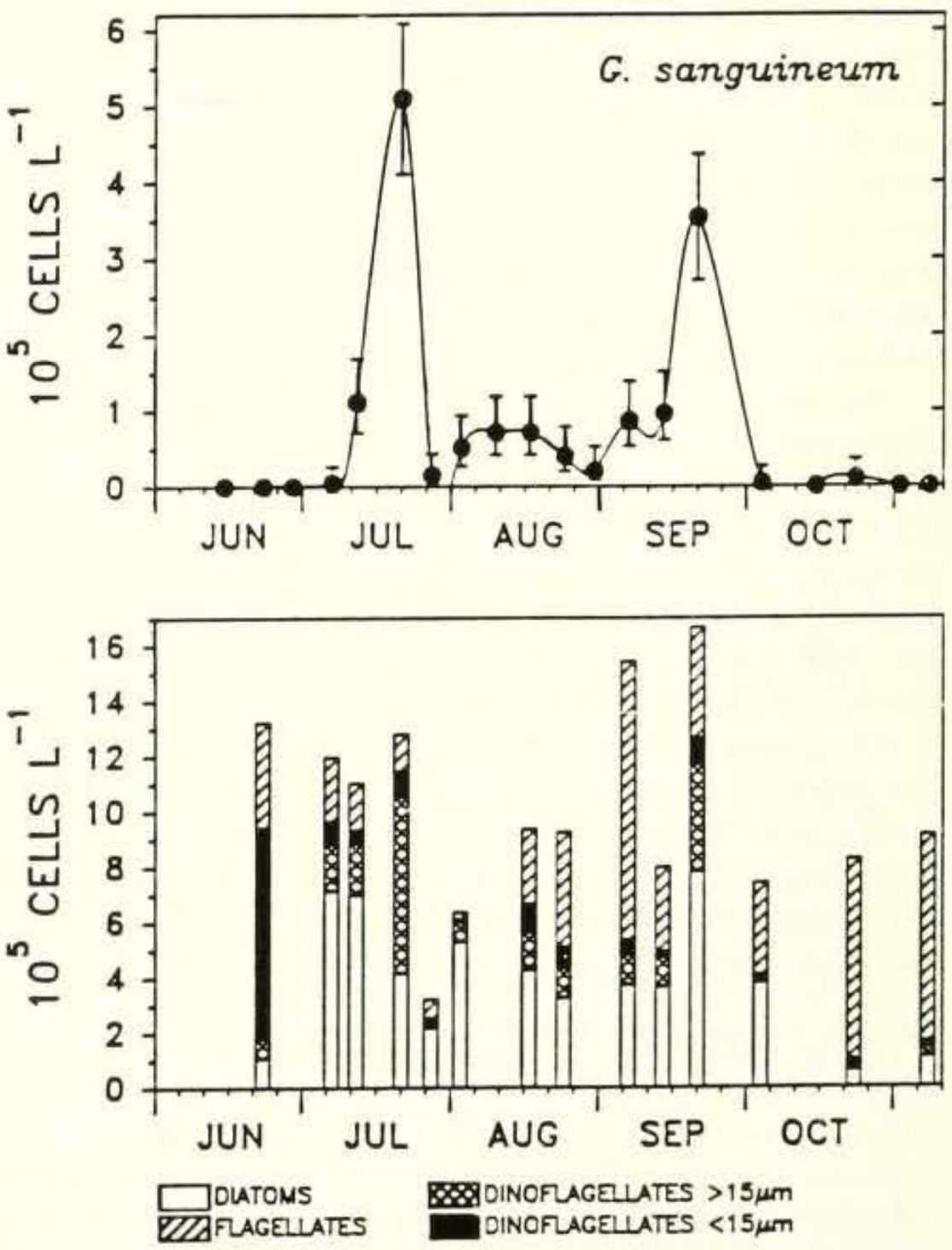



**Figure 3. Phytoplankton composition in Oyster Bay surface water samples during the study period. Top graph: population density (mean and 95% confidence interval) of the dinoflagellate *Gymnodinium sanguineum*. Lower graph: abundance of various phytoplankton groups [*G. sanguineum* is included in the large dinoflagellate (>15 μm) group].**

justed for the lack of independence of samples over time. Because significant differences ($p < 0.05$) were generally found on only one or two of the sampling dates, data from both replicates were pooled.

Small oysters in both density treatments showed highest absolute (slopes of growth curve between sampling dates, Fig. 2) and relative (instantaneous growth coefficient, k, Table 2) shell and tissue growth rates prior to and following the period of maximum mortalities occurring between July 11 and July 26. Thus, during peak mortalities the soft tissue growth coefficient dropped to about ½ of prior and subsequent levels (Table 2). Interestingly, however, growth did not cease during the mortality episode, as small oysters at both densities continued to deposit shell and doubled in tissue weight during the second part of July. Growth slowed again between August 9 and 23, when the lowest growth coefficients were recorded for this experimental group (Table 2). On this date, histological sections of 18 out of 25 SC oysters and 20 of 26 LC oysters had mature gametes, and in some individuals they were present in the gonoducts. A few males showed empty follicles with remnant sperm, indicative of recent spawning activity. Thus reduced growth in mid-August did not coincide with additional mortalities, and was probably associated with spawning activity.

As expected, relative growth of soft tissues was generally higher for small than large oysters (Table 2). Growth patterns of large oysters were similar to those of small oysters, except that reductions in shell and tissue growth rate during early summer occurred two weeks earlier (June 28 to July 11), i.e. prior to, rather than during, the period of heaviest mortalities. As observed for the SC, there was a second period of slow growth during mid August, marked by minimum values in both shell and tissue growth coefficients for oysters held at high density (Table 2), and coinciding with apparent spawning.

In general, temporal patterns of shell growth within a given cohort were very similar between density treatments. Two-way analyses of variance and *a posteriori* multiple comparisons (Sokal and Rohlf 1971) were used to ascertain the effects of date and density on log-transformed shell height. No significant differences were found between density treatments, except for large oysters on July 26 (Fig. 2). Analysis of covariance (ANCOVA, with log height as covariate) was used to examine the effects of date and culture density on log-transformed tissue weights. Among small oysters, the two density treatments were significantly different ($p < 0.05$) on July 11 and July 26, during the mortality episode, whereas among large oysters significant differences were found at most dates (Fig. 2).

The ranges in mean condition index (CI) values over the study period were 77 to 166, and 92 to 127 for the SC and LC, respectively. Differences in condition with stocking density were less pronounced in the SC, and were significant ($p < 0.05$) only on June 28 and July 26 [ANCOVA with log-height as covariate, and multiple comparisons of arcsine (CI/1000) transformed data (Sokal and Rohlf 1971)] (Fig. 4). Condition dropped markedly in July (by 53 and 39% in high and low density groups respectively), at the time of peak mortalities, and again to a lesser extent (30–31% reduction) in mid-August. The mean CI of large oysters stocked at low density was consistently greater than at high density, but showed a similar seasonal pattern (Fig. 4). Significant differences in condition between density treatments were detected on June 28, July 26 and September 6. The greatest decline in condition (9 to 18%) occurred between late June and early July, coincident with early mortalities in this cohort, and a second decline (11 to 16%) occurred in mid-August when growth rates of soft tissues attained a seasonal minimum (Table 2).

The "late cohort" also exhibited considerable shell growth prior to the mortality outbreak, but ceased growing during peak mortalities (Table 2). Mean shell heights (±SE) were 10.3 (±0.2), 14.9 (±0.3), 16.5 (±0.5) and 16.6 (±0.6) on August 2, 9, 16 and 23, respectively.

Production of live oysters, expressed as total volume or weight per unit time, is the most relevant descriptor of performance in a commercial growout operation, and is a function of both growth and survival. Based on scaling considerations, whole body weight and total volume are expected to show comparable rates of increase over time. In the present study whole animal weight, determined in the laboratory, was subject to less measurement error than packed volume, the unit generally used by commercial growers to estimate production, and allowed an estimate of individual variability. Biweekly changes in biomass (total live weight) of the 2 experimental cohorts held at high density declined markedly during the July mortality episode (Fig. 5). Both cohorts experienced the highest production (2.9 to 4.7 increase in biomass over a 2-week period for small oysters, and 2.5–2.8 increase for large oysters) prior to and immediately following the period of mass mortalities. During August and September, the biomass increment over 2 weeks ranged from 1.1 to 1.8-fold, with lowest increments coinciding with declining temperatures (Fig. 1, top graph).

**TABLE 2.**

**Instantaneous growth coefficients for shell height ($k_H$) and dry weight of soft tissues ($k_{DW}$) of large and small experimental oysters held at the high stocking density in Oyster Bay, and $k_H$ of the "late cohort."**

| | Small | | Large | | Late Cohort | |
|---|---|---|---|---|---|---|
| Period | $k_H$ | $k_{DW}$ | $k_H$ | $k_{DW}$ | Period | $k_H$ |
| 6/14–6/28 | 4.27 | ND | 2.44 | 5.54 | 7/26–8/2 | 4.28 |
| 6/28–7/11 | 3.24 | 9.07 | 0.35 | 3.18 | 8/2–8/9 | 5.23 |
| 7/11–7/26 | 1.24 | 4.32 | 1.88 | 6.69 | 8/9–8/16 | 1.48 |
| 7/26–8/9 | 3.52 | 10.17 | 2.04 | 7.82 | 8/16–8/23 | 0.05 |
| 8/9–8/23 | 0.62 | 1.68 | 0.76 | 0.69 | | |
| 8/23–9/6 | 1.50 | 4.80 | 0.74 | 2.74 | | |
| 9/6–9/20 | 0.78 | 2.70 | 0.90 | 2.74 | | |

$k = ([\ln x_2 - \ln x_1]/t) \times 100$, where $x_2$ and $x_1$ are final and initial heights (mm) or weights (mg) respectively, and t = time interval. ND = not determined; date notation as in Table 1.

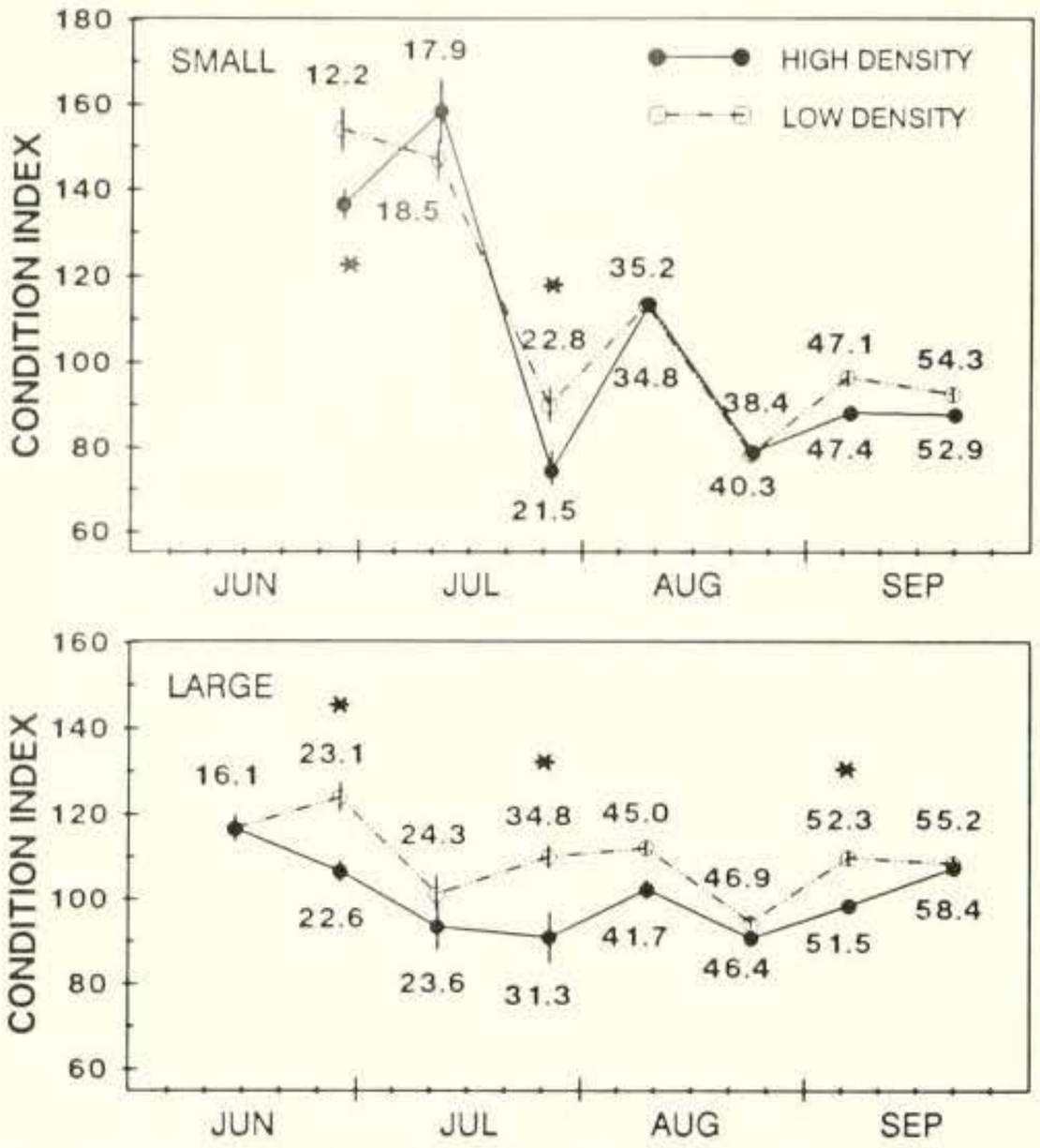



**Figure 4. Mean gravimetric condition index (see methods) (±SE) of large and small oyster cohorts cultured at 2 stocking densities in Oyster Bay. Mean shell heights are indicated at each sampling date; asterisks indicate significant differences ($p < 0.05$) in mean condition between density treatments.**

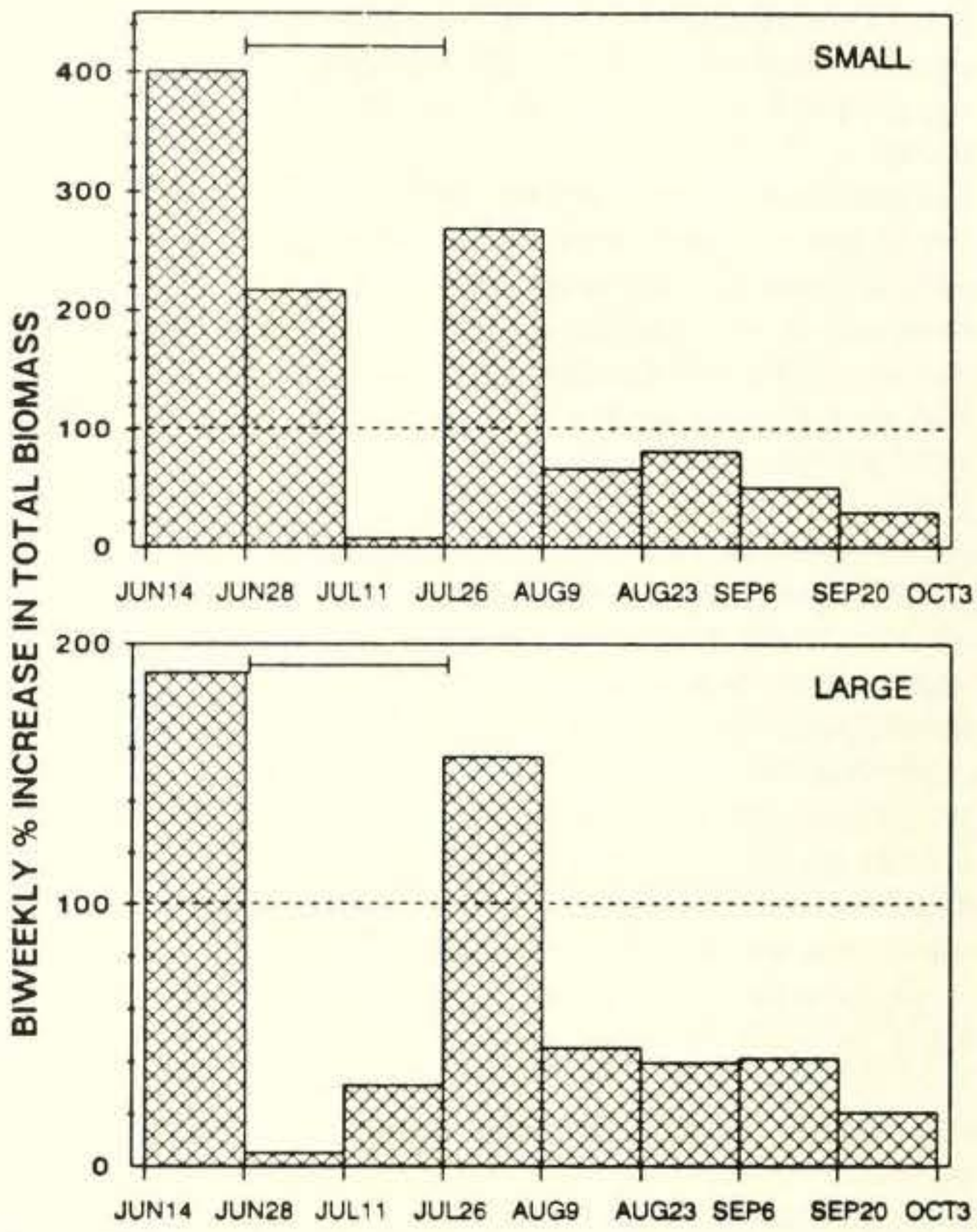



**Figure 5. Percent biweekly change in total live biomass of small and large oyster cohorts held at the high stocking density in Oyster Bay, calculated as: $[(n_2W_2 - n_1W_1)/n_1W_1] \times (t_2 - t_1)/15 \times 100$, where $n_1$ and $n_2$ = numbers of survivors for each sampling interval $(t_2 - t_1)$ = 13 to 15 days, $W_1$ and $W_2$ = mean whole body weight of live oysters (pooled data from 2 replicate experimental groups, n as in Fig. 2). Horizontal dashed lines indicate the level corresponding to a doubling of biomass over a 2-week period, and solid horizontal bars mark the period of mortalities. Initial n was arbitrarily selected as that initially deployed in one experimental tray (35,000 and 7,200 oysters for small and large cohorts respectively); 100% survival was assumed after the July 26 sampling date.**

### Shell Anomalies

Although most juvenile oysters examined had mats of bacteria and other microorganisms on the external shell surface, gross and histological examination of the shell indicated no evidence of penetration by fungus or other shell-boring organism.

Macroscopically, the most consistent correlate with the juvenile oyster mortality syndrome, in both living and dead animals, was a layer of abnormal conchiolin deposited on the inner surface of one or both valves, but primarily on the left valve. It was frequently raised into a ridge several millimeters from the edge of the shell (Fig. 6, right valve). Most often, the ridge formed a completely closed ring (Fig. 6, right valve) on only one valve; however, rings were found on both valves of some oysters. It was not unusual to find the ridge juxtaposed to the adductor muscle along the dorsal to posterior margin of the muscle (Fig. 6, right valve). In some cases, the conchiolin layer was deposited between the adductor muscle and the shell, causing the muscle to detach. Tissues of live oysters were usually found contracted within the bounds of the ridge; however, in some cases the ridge was present inside the free edge of the mantle. Portions of the shell external to the conchiolin ridge were frequently covered by mud and fouling organisms. Some oysters, apparently in early stages of the syndrome, were found with the thin conchiolin sheet covering all or only small portions of the shell surface, but with no ridge. The left valve of affected oysters was often deeply cupped, its edge extending beyond that of the right valve.

Prevalence of abnormal conchiolin increased markedly during July in concert with the increase in mortalities (Fig. 7). Small oysters in both density treatments showed similar patterns, such that 43–48% of living oysters exhibited the syndrome by late July, after which prevalence decreased to nearly zero. In contrast, LC oysters showed a relatively high prevalence (24–34%) as early as July 11. The presence of abnormal conchiolin decreased thereafter in the low density treatment, but remained high (40%) in the high density animals through the end of July, after which it declined (Fig. 7). Prevalence of abnormal conchiolin was also high in the "late cohort," with increasing values of 21%, 40% and 52% on August 9, 16 and 23 respectively.

Longitudinal cross-sections of the lower (cupped) valve of 15 survivors from the SC collected on September 20 were examined for evidence of past alteration in the pattern of shell deposition. No anomalous deposition was apparent on the external surface of the shell or in cross-section, suggesting that survivors were relatively unaffected at the time of mass mortalities.

### Histopathology

Light microscopy of samples collected during peak mortality showed that oysters depositing abnormal conchiolin possessed lesions of the mantle characterized by degeneration and sloughing of epithelial cells, infiltration of hemocytes into epithelium and un-

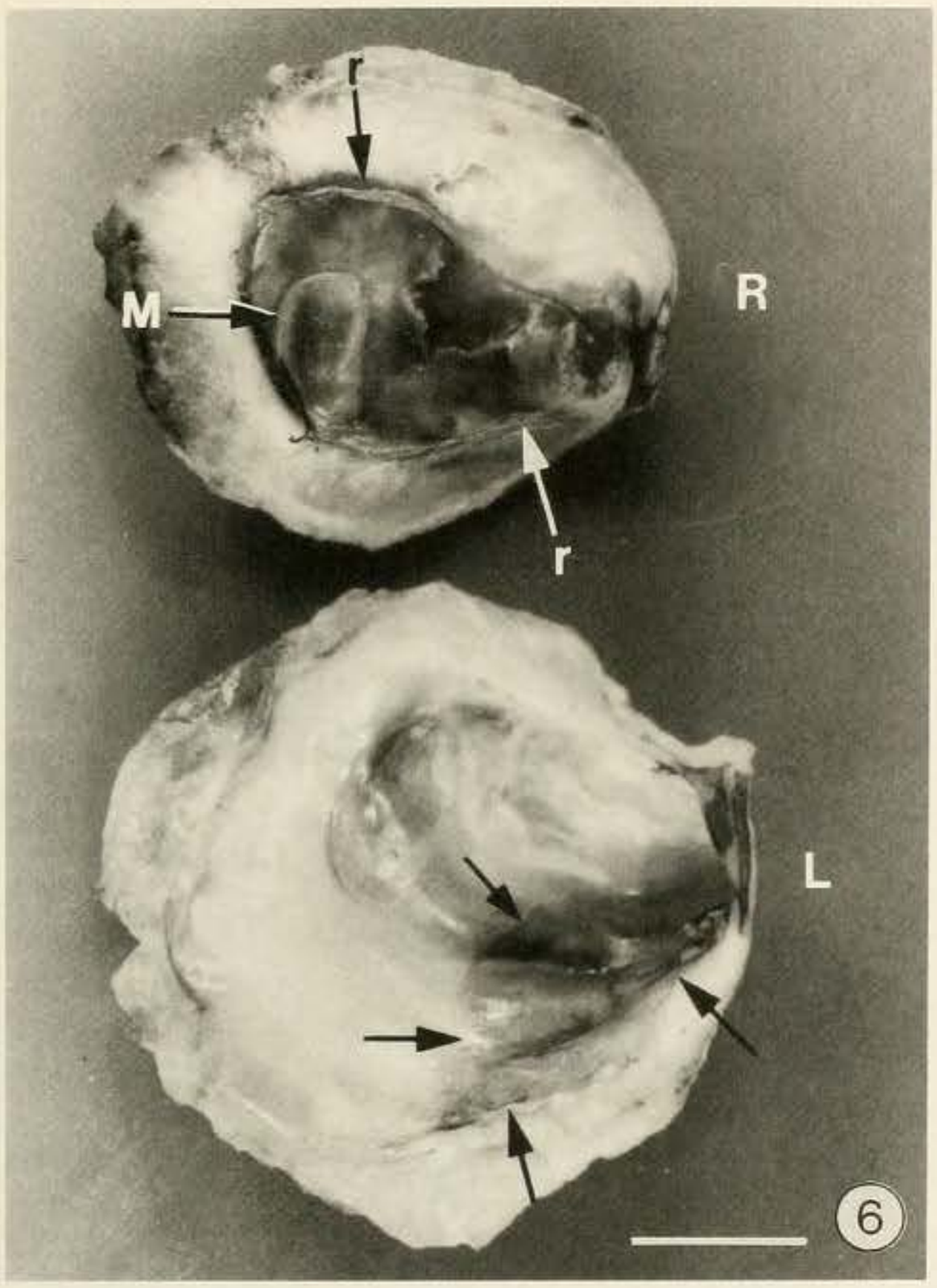



**Figure 6. Right (R) and left (L) valves of juvenile oyster where anomalous conchiolin deposits have been formed on the inner shell. The right valve has a conchiolin deposit organized into a peripheral ridge (r) and a thin layer covering the shell within the ridge except where the adductor muscle (M) was attached. Conchiolin on the left valve is layered onto the shell within the area denoted by arrows. The ridge was formed by the mantle beneath and just inside the leading edge of the free mantle and just inside where the visceral mass was in contact with the shell. Bar = 4 mm.**

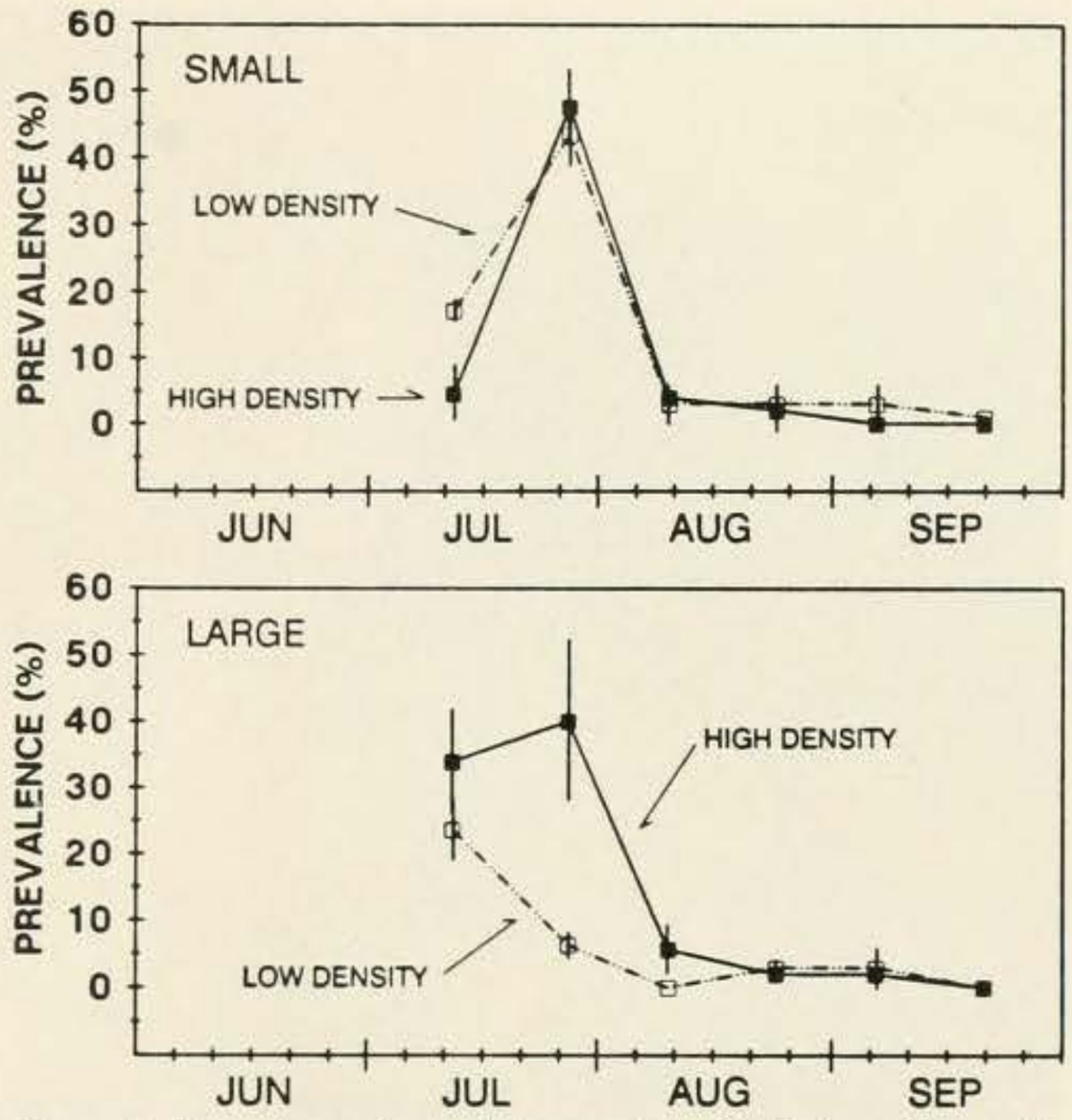



**Figure 7. Percent prevalence of abnormal conchiolin deposition on the inner surface of the cupped (left) valve and/or right valve (see Fig. 6) in small and large live, experimental oyster cohorts held at 2 stocking densities in Oyster Bay (prevalence was not determined prior to July 11). Error bars and sample size as in Fig. 2.**

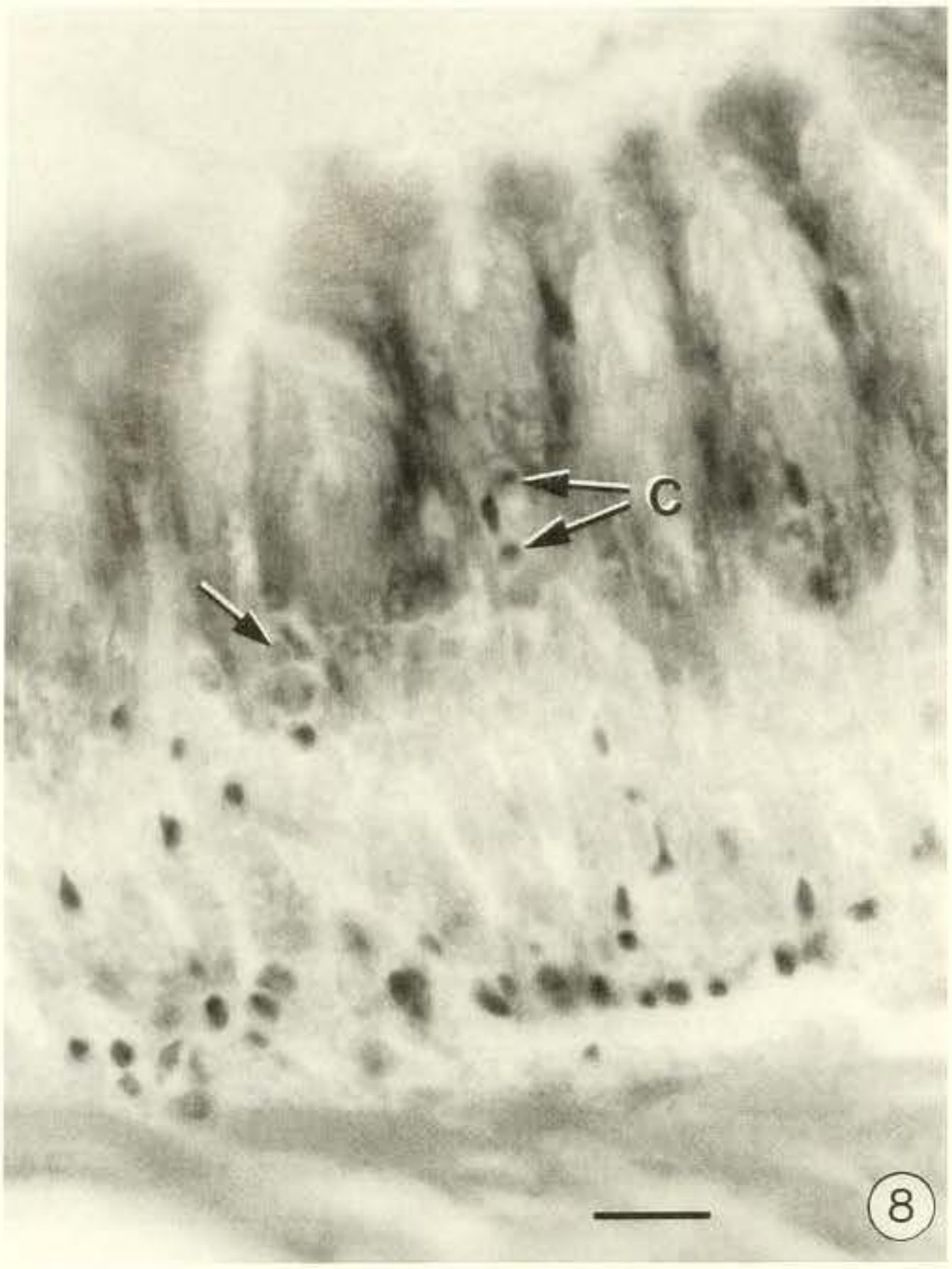
derlying connective tissues, and the presence of dense coccoid bodies within phagocytes and epithelial cells (Fig. 8). These bodies often contained one or more punctate, basophilic structures that resembled nuclei of some eucaryotic microbes (Figs. 8 and 11); however, the bodies had a wide range of sizes (<1 to 6 μm) and many contained no basophilic structures (Figs. 8 and 10). Bacteria and ciliates were present in some lesions where the epithelial layer had been eroded and were common in moribund oysters. Degeneration of the myoepithelium was observed in some oysters, accompanied by muscle degeneration and detachment from the shell. Hemocytes, bacteria, and debris were found against both sides of the conchiolin layer and within chambers and spaces between sublayers of the conchiolin (Figs. 9 and 12). The connective tissues in the visceral mass and the digestive epithelia were not affected, and food was present in the guts of most oysters. Hemocyte infiltration and epithelial degeneration were found in the gills of some individuals. No recognizable organism was present consistently in all lesions and all oysters.

Lesions were present, but less severe, in oysters categorized macroscopically as having few or no signs of distress. The smallest, and presumably earliest, lesions consisted of disruptions to the apical portions of epithelial cells under the hinge ligament and in the pallial (peripheral) region of the mantle. Infiltration of hemocytes into the adjacent connective tissue appeared in more advanced cases, followed by epithelial sloughing.

Transmission electron microscopy of mantle tissue from diseased oysters showed that the coccoid bodies seen in light microscopy consisted of portions of oyster cells within phagocytes and epithelial cells, or free in spaces between epithelial cells where they were mixed with cell debris. The substructure of these bodies included mitochondria that resembled those of oyster cells (i.e., having shelf-like cristae), lipoid droplets, concentric arrays of rough endoplasmic reticulum, and electron-dense granular material, delimited by two membranes, which could be chromatin of pycnotic nuclei (Fig. 11). We believe that the latter structures are the basophilic parts of the bodies seen by light microscopy (Fig. 8). When found in whole cells, the coccoid bodies were delimited by a vacuolar membrane. Myelin whorls (Fig. 10) and multivesicular bodies were also characteristically found in the cells containing the coccoid bodies. Most probably these bodies are secondary lysosomes resulting from an extreme example of autophagy. Thin sections of anomalous conchiolin showed bacteria present in the chambers. All of the bacteria had walls characteristic of gram negative species. Some were rods (Fig. 12) and others were filamentous.

### Temporal Development of Lesions

No mantle lesions were observed in the initial sample of SC oysters collected on June 16, but they were present in 30% of the sample by June 28 and 83% by July 11 during the height of mortality (Fig. 13). When deaths ceased in late July, nearly 70% of survivors still showed lesions, but they decreased to 16% by



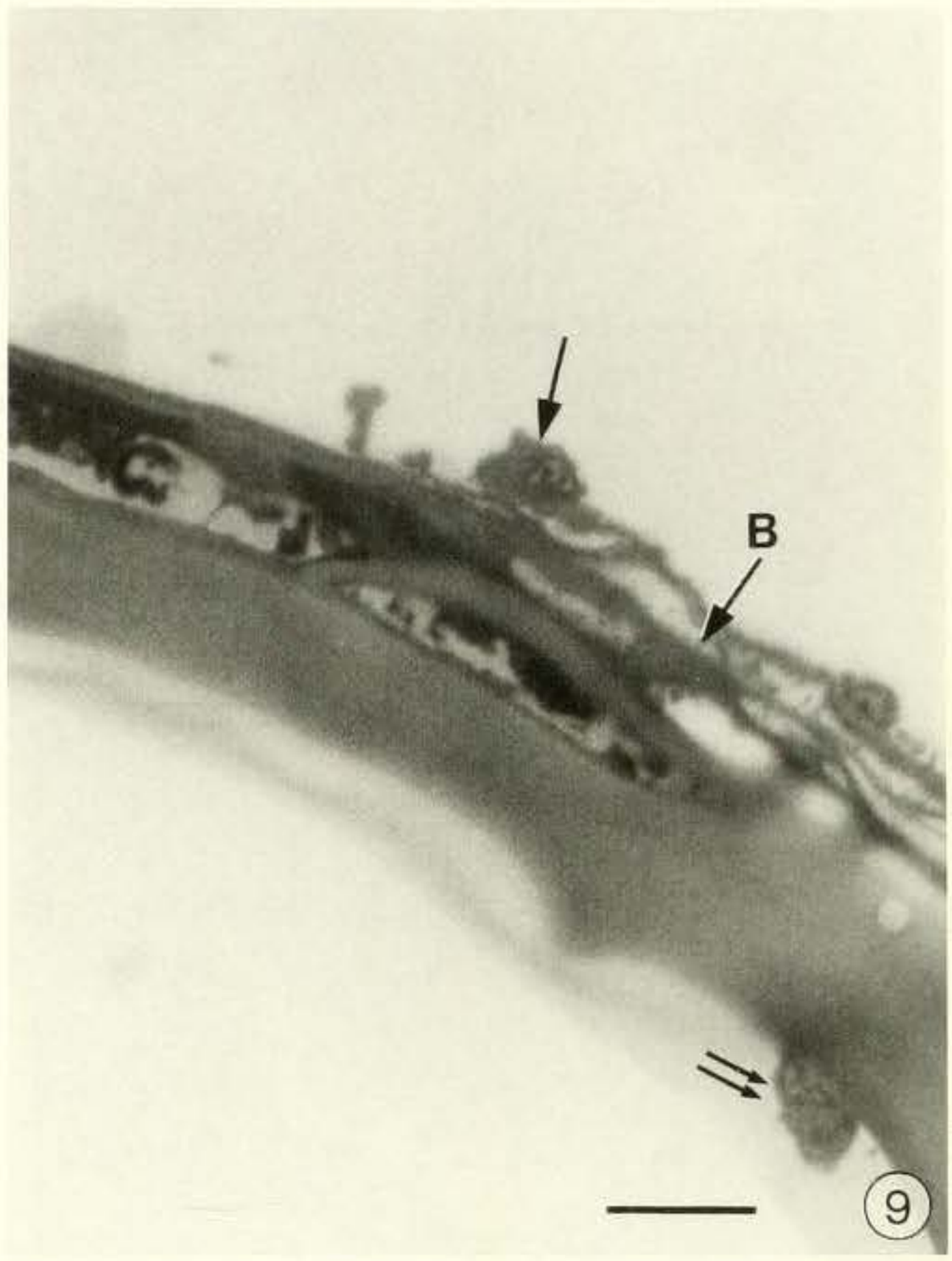




**Figure 8. Hematoxylin and eosin-stained histological section of the mantle of a juvenile oyster in early stages of anomalous conchiolin formation. The mantle sample was obtained from the posterior region of the oyster and was found overlying a ring of anomalous conchiolin. Coccoid bodies in mantle epithelium with (C) and without (arrow) basophilic substructure. Bar = 10 μm.**

**Figure 9. One-half micron, resin-embedded section of anomalous oyster conchiolin. Oyster hemocytes on what is believed to be the outer surface (arrow) and inner surface (double arrow) of the conchiolin deposit; bacteria in chambers (B). Bar = 10 μm.**

August 9. In contrast to the SC, lesions were already present in 40% of the LC on June 16. Two weeks later, prevalence was 80%, a figure maintained until July 11. Thereafter, prevalence decreased, reaching a low of 40% of August 9. Interestingly, mantle lesions became more frequent in late September, being found in 60–70% of both groups, before decreasing again in early November (Fig. 13). The prevalence of moderate to heavy lesions was greater than 10% only during the period of heaviest mortality on July 11, and in September 20 samples when lesions were found in digestive, as well as mantle, epithelia.

Oysters examined at Battelle had a somewhat different percentage of lesions, but the pattern was similar. Lesions began to appear in the inner lobe of the mantle and digestive epithelia in September and persisted in the latter through the final samples collected in mid-November.

The prevalence pattern of coccoid bodies mirrored that of the lesions, although they were found in only about half of oysters in which lesions were present (Fig. 13). Bacteria and ciliates were most frequent in late June and July samples, when they were found in up to 30% of the oysters.

### *Sampling at the Fishers Island Study Site*

At Fishers Island only the SC experienced heavy mortalities (up to 96% between July 10 and July 25), whereas cumulative mortality of the LC was only 8 to 11% by the end of the study (Table 3). The number of survivors from the SC was insufficient to continue sampling after July 25.

During early July, when mortalities first started, small oysters exhibited tissue weight loss (27 and 43% reduction over two weeks in high and low density treatments, respectively), and cessation of shell growth. In contrast, large oysters continued to grow at an accelerated rate in both shell and soft tissues during this period ($k_H$ = 1.35 and $k_{DW}$ = 4.92 between June 27 to July 10), but experienced no shell growth and inhibition in soft tissue growth ($k_{DW}$ = 1.43) at the time the SC suffered heaviest losses (July 10 to 25) (Table 3).

Three-quarters of the SC collected on July 10, when mortalities were beginning, had mantle lesions similar to those seen at the Flower Co. Coccoid bodies and bacteria/ciliates were found in 30% and 60%, respectively, of the sample. At the same time, only 20% of the large cohort had lesions and 5–10% showed coccoid bodies, bacteria, or ciliates.

## DISCUSSION

At the Oyster Bay site, severe mortalities successively affected several 1991 cohorts of juvenile oysters during July and August, within 3 to 6½ weeks of transfer from the hatchery to the field growout system. Mortalities appeared to be species-specific, since

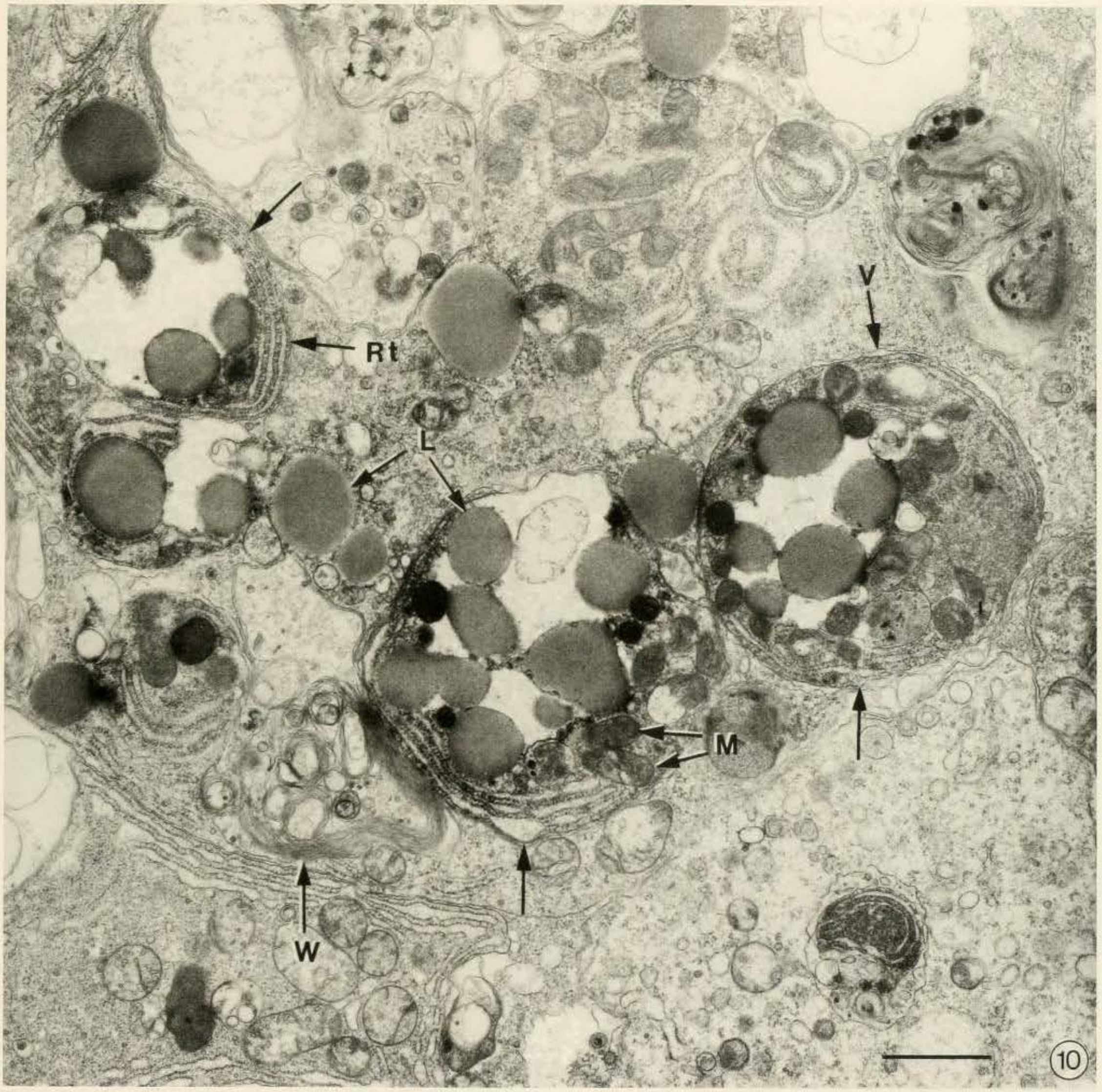



**Figure 10. Cytoplasm of mantle epithelial cell from region overlying anomalous conchiolin deposit. Coccoid bodies (arrows) within epithelial cell; lipoid droplets (L); mitochondria (M); rough endoplasmic reticulum (Rt); myelin whorl (W), and membrane of vacuole containing coccoid body (V) Bar = 1 μm.**

juvenile hard clams, *Mercenaria mercenaria*, held in adjacent stacks in the same growout system, were not affected. Cumulative mortalities reached 54 to 76% in animals held at commercial densities, and affected animals ranging between 15 mm (late cohort) and 24 mm (LC) in mean shell height at the time of initial losses.

The three experimental cohorts from the Flower's hatchery included in the present study originated from common, Oyster Bay broodstock, although different individuals were spawned to produce each cohort. Juvenile oyster mortalities occurred again at this site in the summer of 1992, with progeny produced from wild broodstock from the Thames River, Connecticut (Borrero and Ford, unpubl. data). Furthermore, seed produced from Oyster Bay broodstock suffered no unusual mortalities when reared at the Bluepoints hatchery, on the south shore of Long Island, in 1992. The causes of mortality thus appear to be site-specific and unrelated to broodstock origin.

Heavy mortalities of juvenile oysters from a different broodstock were also documented at the Fishers Island growout site over a 2-week period in July 1991. Only small cohort oysters were affected, within 4 weeks of deployment, when individuals averaged 12 mm in shell height. Although mortalities coincided at Oyster Bay and Fishers Island, it is not clear at this time that they can be linked to a common cause. Since a more extensive data

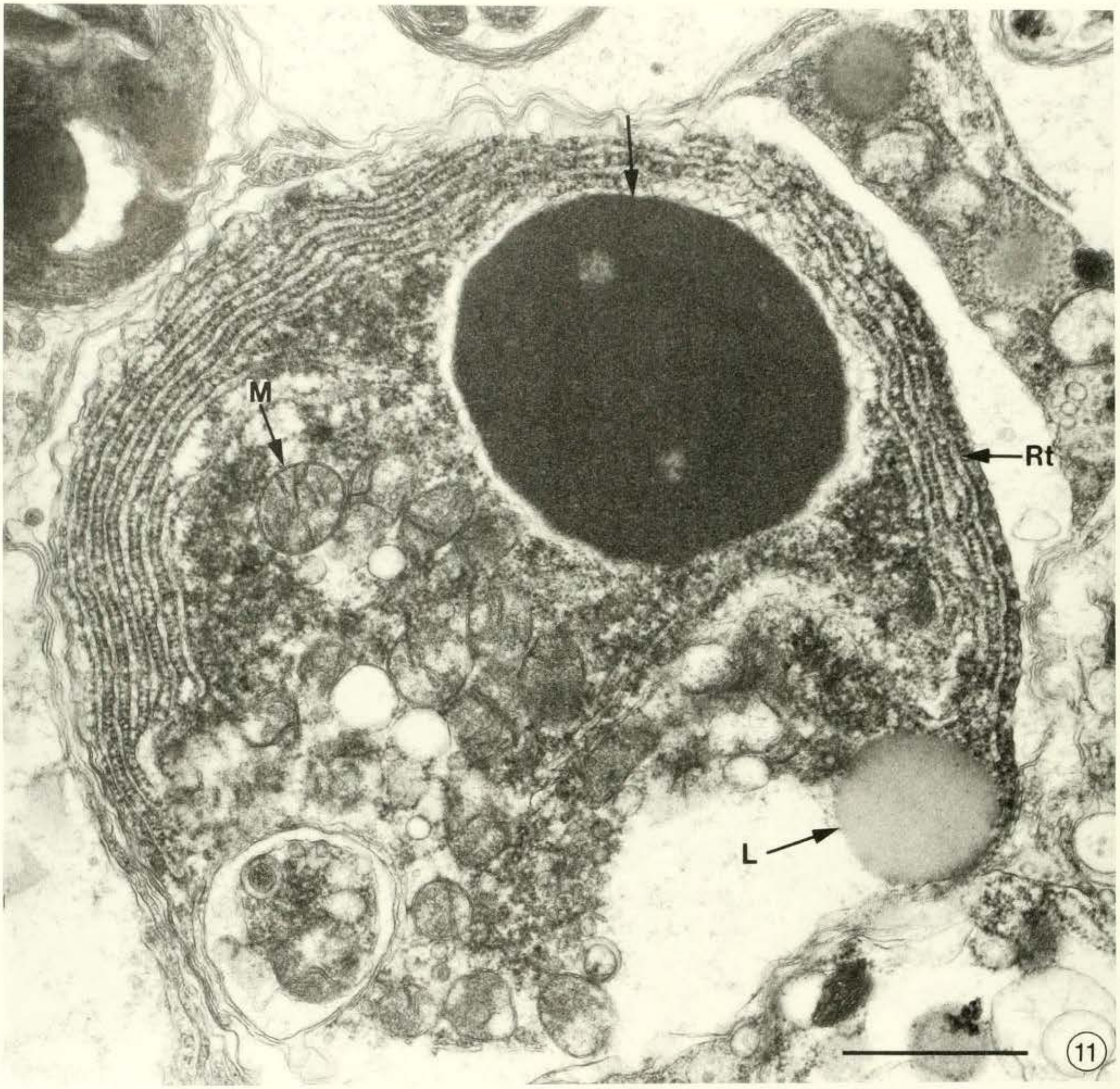



**Figure 11. Coccoid body from the same tissue region as in Fig. 10. Electron dense granule (arrow) is believed to be degenerate chromatin material which is the same as the basophilic structures of coccoid bodies when seen in light microscope sections (see Fig. 8). Rough endoplasmic reticulum (Rt); mitochondria (M); lipoid droplet (L). Bar = 1 μm.**

base is available for oysters grown in Oyster Bay, our remaining discussion will focus on findings at this site.

***Potential Role of Food Limitation and Toxic/Noxious Microalgae***

The progression of oyster mortalities in Oyster Bay was remarkably fast, spanning a period of only 2 to 4 weeks. Surprisingly, oysters continued to grow in both shell and tissue weight at this site, albeit at a reduced rate, during the mortality episodes. Positive growth of the population, however, as reflected in mean values, may mask differences in growth and survival among individuals with varying susceptibility to the stressor involved. Visual examination of gut coloration and tissue slides indicated that animals had relatively full alimentary tracts throughout the study. Also, juvenile bivalves are known to survive fairly prolonged starvation [e.g. 10 mm *Mercenaria mercenaria* experienced no mortalities and only 9% dry tissue weight loss when held in filtered seawater in the laboratory for 4 weeks at 24–25°C (Bricelj and Borrero, unpubl. data)], and can minimize weight loss under starvation through a reduction in metabolic rate (Bayne 1973, Malouf and Breese 1978). The above findings therefore suggest that the mortalities documented are unlikely to have resulted from starvation. Dominance in the seston of phytoplankton species of poor nutritional value [e.g. chlorophytes such as *Nannochloris atomus* and *Stichococcus* sp. (Bass et al. 1990)] at high summer temperatures could, however, induce feeding activity, maintain high met-

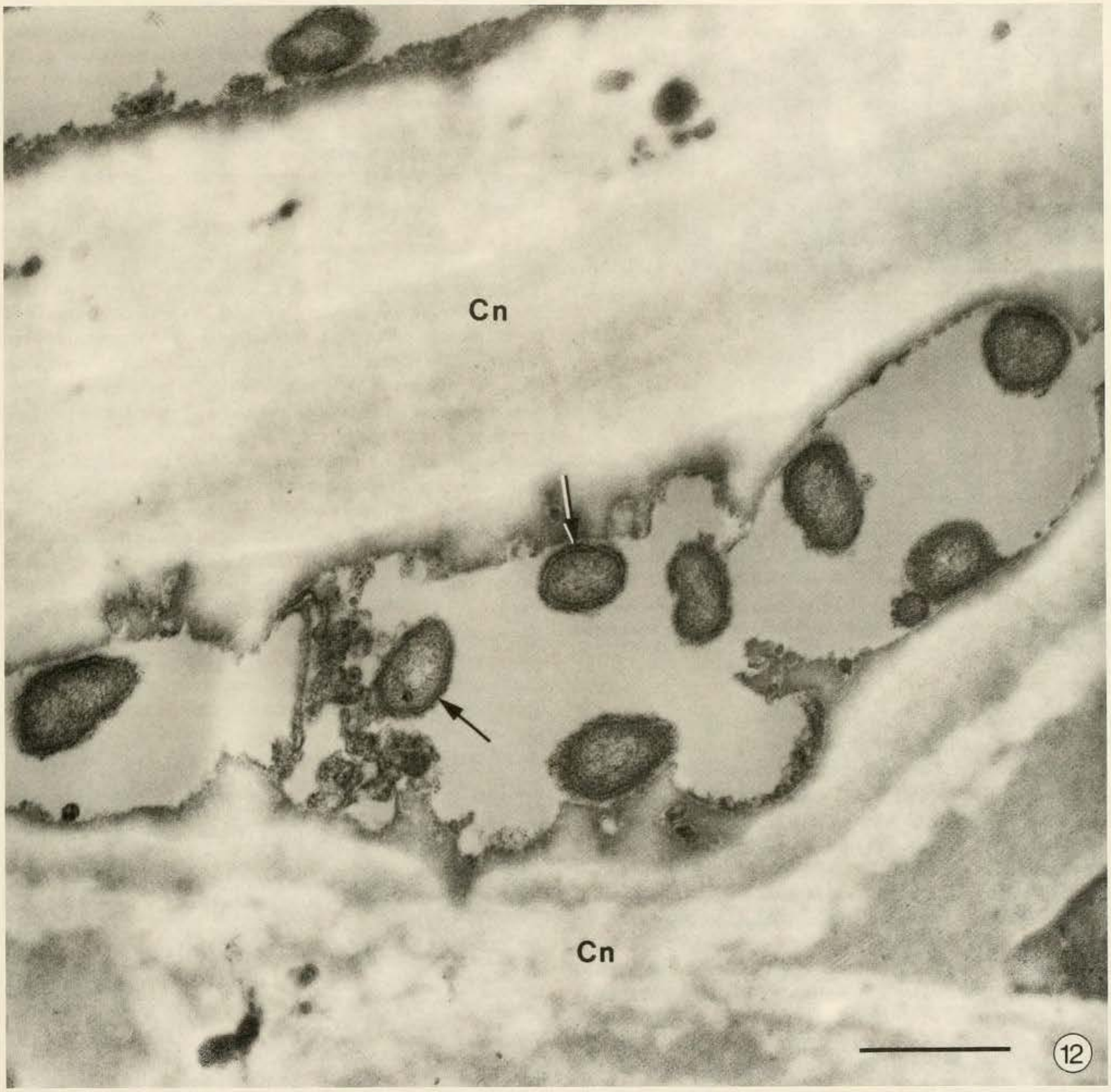



**Figure 12. Chamber between layers of anomalous conchiolin (Cn) in which gram negative bacteria (arrows) are present. Bar = 0.5 μm.**

abolic rates, and lead to greater weight loss than the absence of food. The time-frame of mortalities and the fact that oysters did not experience weight loss prior to, or during, the period of mortalities, are thus more compatible with stress effects induced by a water or particle-borne contaminant, pathogen and/or the effect of noxious/toxic algae, than food limitation *per se*.

Blooms of the picoplanktonic alga, *Aureococcus anophagefferens*, which is known to cause starvation and mortalities of bivalves (Bricelj et al. 1987, Tracey 1988), have never been recorded in Long Island Sound or adjacent estuaries along the north shore of Long Island. However, a bloom of *Gymnodinium sanguineum* (Dinophyceae) was documented at the Oyster Bay grow-out site between July 11 and July 26, coinciding with peak mortalities of 2 of the experimental cohorts. Cell densities of this dinoflagellate species remained relatively high (at ca. $5 \times 10^4$ cells $l^{-1}$) during early August when the "late cohort" experienced mortalities. No mortalities were associated with a second bloom in September, and only the small cohort in the high density treatment showed a reduction in shell and tissue growth at this time (Table 2). It is noteworthy, however, that the prevalence of mantle lesions and coccoid bodies increased again in both cohorts between August 9 and September 20 (Fig. 13), when dinoflagellate concentrations attained a second maximum. On the other hand, mantle lesions were prevalent in late June, before the increase in *G. sanguineum* cell numbers. Ingestion of toxic dinoflagellates has in some cases been associated with cellular damage (e.g. abnormal vacuolation) of the digestive epithelium in bivalves (Widows et al. 1979, Wikfors and Smolowitz, in press). In the present study

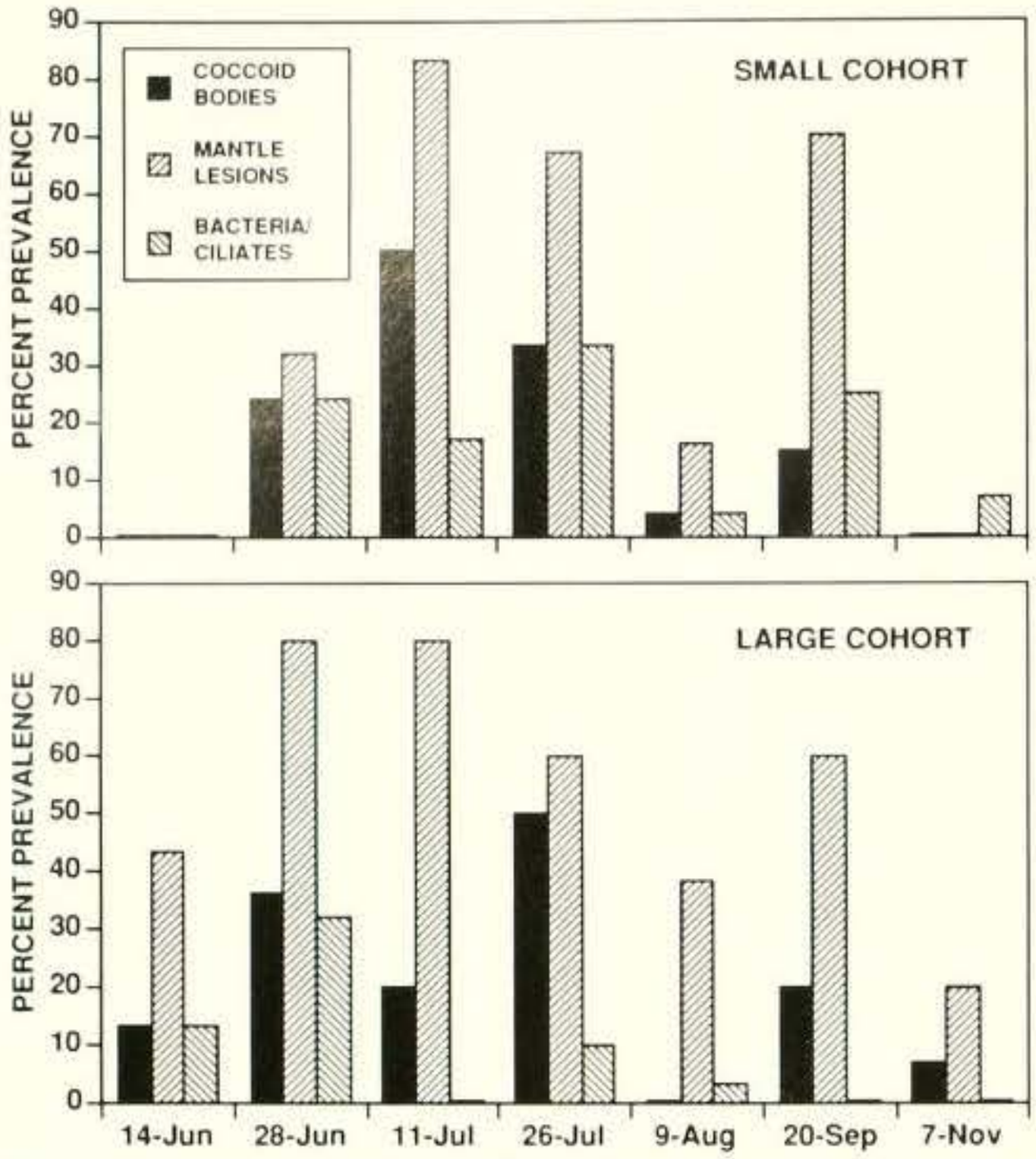



**Figure 13. Percent prevalence of 3 pathological conditions observed by light microscopy in tissue sections of juvenile oysters collected at Oyster Bay.**

lesions in the digestive gland were observed only in oysters collected in late September and thereafter.

Although *G. sanguineum* has not been previously reported to be toxic to marine fauna, other dinoflagellate species common to east coast estuaries in the summer, are toxic to and cause mortalities of bivalves, including oysters (reviewed by Shumway 1990 and Shumway et al. 1990). The eastern oyster, *Crassostrea virginica*, is particularly susceptible to toxic dinoflagellates. For example, one week's exposure to bloom concentrations of the unarmored dinoflagelate *Gyrodinium aureolum*, (a species closely related to *G. sanguineum*, and associated with fish and shellfish kills [reviewed by Mahoney et al. 1990]), caused 68% mortalities in juvenile *C. virginica* at 10°C, 6 weeks after exposure (Shumway, unpubl. results). Similarly exposed hard clams, *M. mercenaria*, experienced no mortalities. Oysters were able to filter this alga from suspension and were the most affected of 8 bivalve species tested. *Gyrodinium aureolum* was also implicated in mortalities of softshell clams and mussels in Maquoit Bay, Maine, in 1988 (Heinig and Campbell 1992). A bloom of the closely related toxic species *Gymnodinium breve* (*Ptychodischus brevis*) in North Carolina in 1987 resulted in recruitment failure of bay scallops (Summerson and Peterson 1990) and brevetoxin accumulation in oysters and hard clams (Tester and Fowler 1990). The dinoflagellate *Prorocentrum minimum*, which occurs in Long Island Sound, appears to be toxic to some bivalve species, such as the bay scallop, *Argopecten irradians* (Wikfors and Smolowitz, in press).

Early life history stages of bivalves are generally more susceptible to the detrimental effects of toxic algae. Thus, a summer bloom of *Gyrodinium* cf. *aureolum* (= *Gymnodinium nagasakiense*) in the Bay of Brest, France, caused heavy mortalities of postset scallops, *Pecten maximus* (0.25–3 mm), but only cessation of growth and shell abnormalities of juveniles (5–30 mm) held in a shore-based nursery, and growth disturbance rings in wild adults on the bay bottom (Erard-Le Denn et al. 1990). In conclusion, the large biomass contribution of *Gymnodinium sanguineum* at the time of oyster mortalities in Oyster Bay, and the fact that no information is available on this species' toxicity to *C. virginica*, raises the possibility that mortalities could have been caused by noxious/toxic phytoplankton. If so, it is not yet clear why no oyster mortalities were associated with the second bloom of *G. sanguineum*.

### *Role of Physical Environmental Factors*

Environmental factors such as temperature, salinity, and low levels of oxygen can interact synergistically with other stressors. In the present study, however, salinities and temperatures remained within normal levels for this site, although earlier spring warming occurred in 1991 relative to the 4 previous years. The water column is relatively shallow and well mixed, thus precluding oxygen limitation, except in an anoxic microzone that developed within the trays around dead animals. Dense algal blooms could, however, cause transient hypoxia during night hours, when the oxygen demand may exceed supply (see discussion in Heinig and Campbell 1992). Oyster mortalities of three experimental cohorts cultured at this site were restricted to the months of July and August. However, the "late cohort" was the last produced at this study site in 1991; there is no way of determining whether abnormal mortalities would also have affected later cohorts held in the system during the early fall period of decreasing temperatures. Earlier oyster cohorts held in the same growout system, but not tracked by our study, suffered heavy mortalities as early as July 4, when the water temperature was about 22°C. However, the earliest cohort, originating from a spawning in late February, which was deployed in the trays in the first week of April, first reached commercial bottom planting size (20–30 mm) on June 17 without experiencing anomalous mortalities (D. Relyea, Flower Co., pers. comm.).

Dinoflagellate blooms tend to occur during the warmer months of the year. Chang and Carpenter (1985) found that temperature was the principal factor controlling the appearance of summer blooms of *Gyrodinium aureolum* in the Carmans River estuary, Long Island. The rapid decline in *G. sanguineum* cell densities in Oyster Bay in late September, once temperatures dropped below ca. 18°C, may also be temperature related. Thus, while the exact role of elevated summer temperatures in relation to the oyster mortality events cannot be ascertained from our data, we suggest that they may have played an indirect role (e.g. through control of phytoplankton species composition and abundance or microbial activity) in the development and/or progression of mortalities, since these were not documented until temperatures exceeded about 22°C.

### *Comparative Response of Large and Small Cohorts*

The two main cohorts available for comparison at this site, differed by only about 10 mm in mean shell height at the time of deployment. Thus size-dependent mortality can only be assessed over a relatively narrow size range.

In both experimental cohorts deployed on June 14, mortalities coincided with: a) reduction in shell and soft tissue growth (Table 2), b) reduction in gravimetric condition index (Fig. 4), and c) increased prevalence of a distinct ring of conchiolin deposited on the inner shell surface (Fig. 6) and of mantle lesions (Fig. 13).

**TABLE 3.**

**A) Surface water temperature and mean percent cumulative mortalities (SD = standard deviation, n = 2 replicates) of large and small oyster cohorts cultured at Fishers Island at high and low stocking densities (see Table 1). B) Mean shell height ($\overline{H}$, mm) and dry soft tissue weight ($\overline{DW}$, mg) obtained by pooling data from two replicate experimental groups at the high-density treatment [data only available for some of the sampling dates (see text)]. ND = not determined; SE = standard error, n = sample size.**

A) % Cumulative Mortality Mean (SD)

| | | Small Cohort | | Large Cohort | |
|---|---|---|---|---|---|
| **Date** | **T (°C)** | **High** | **Low** | **High** | **Low** |
| 6/12 | 18 | 0.9 | 0.9 | 8.8 | 8.8 |
| 6/27 | 18 | 0 | 0 | 5.4 (5.4) | 5.2 (0.6) |
| 7/10 | 18 | 15.6 (11.6) | 9.0 (5.6) | 2.8 (1.9) | 1.8 (2.6) |
| 7/25 | 20 | 96.4 (1.1) | 100 | 6.8 (6.0) | 4.2 (1.2) |
| 8/8 | 22 | | | 6.0 (1.9) | 6.2 (1.2) |
| 8/29 | ND | | | 6.8 (1.5) | 3.3 (2.0) |
| 9/13 | 21 | | | 7.1 (1.0) | 3.6 (0.3) |
| 9/26 | 18.5 | | | 10.8 (2.0) | 8.2 (3.0) |

B) Mean Shell Height ($\overline{H}$) and Tissue Dry Weight ($\overline{DW}$)

| | Small Cohort | | | | Large Cohort | | | |
|---|---|---|---|---|---|---|---|---|
| **Date** | **$\overline{H}$** | **(SE, n)** | **$\overline{DW}$** | **(SE, n)** | **$\overline{H}$** | **(SE, n)** | **$\overline{DW}$** | **(SE, n)** |
| 6/12 | 8.9 | (0.2,49) | 2.54 | (0.13,49) | 31.7 | (5.0,30) | 75.40 | (4.32,30) |
| 6/27 | 12.2 | (0.3,60) | 7.26 | (0.46,59) | 33.4 | (0.7,60) | 120.89 | (5.23,60) |
| 7/10 | 12.0 | (0.3,56) | 5.28 | (0.44,39) | 39.8 | (0.7,60) | 229.24 | (12.01,60) |
| 7/25 | | | | | 38.9 | (1.0,60) | 283.98 | (15.98,60) |
| 8/8 | | | | | 44.2 | (0.8,98) | 280.38 | (13.28,98) |
| 9/13 | | | | | 53.9 | (1.1,56) | ND | |

Several differences in response were observed, however, between the two cohorts. It is noteworthy for example, that the LC showed detrimental effects on growth, and appearance of the abnormal conchiolin syndrome and mantle lesions 2 weeks earlier than the SC. This correlates with the appearance of mortalities 2 weeks earlier than in the SC. The drop in condition index, coincident with mortalities, however, was much more pronounced in the SC (53% compared to only 9% in high-density treatments), yet mortalities peaked at similar levels in both cohorts by July 26. Galtsoff (1964) suggested that young *Crassostrea virginica* have flatter valves and therefore a higher CI than larger oysters (Galtsoff 1964). Rainer and Mann (1992) found no size-dependency of the volumetric CI in *C. virginica*, but tested this only for oysters 36 to 96 mm in height. The difference in initial CI values observed between cohorts in the present study cannot be attributed solely to size-dependency, however, since SC oysters had lower values even when they attained the same size as LC oysters (compare the mean condition index for the SC on July 11 with that of the LC on June 26 in Fig. 4). Difference in mortalities, growth and condition between the 2 cohorts are thus not clearly correlated with size, and may simply reflect inter-batch variability.

A decline in condition represents a sensitive but generalized stress response, of limited value in inferring specific causes of mortality. Reductions in the condition index of oysters have been related to reproductive condition (e.g. Nascimento and Pereira 1980), nutritional stress (Wright and Hetzel 1985), as well as disease (Newell 1985, Paynter and Burreson 1991). Interpretation of changes in the condition index can be confounded if rates of growth in shell and soft tissues are uncoupled (Hilbish 1986). In the present study, however, reductions in growth coincidentally affected both shell and soft tissues.

Mean tissue weight and condition index were generally greater at the lower stocking density, although these differences were not always statistically significant, especially for the SC. Differences in tissue weight between density treatments averaged only 21–24% over the study period in both cohorts, and were more pronounced during the early part of the experiment (through July 26). Mortalities, however, were reduced by as much as 39–45% at the low stocking density. Thus, reduced densities had a greater effect in reducing mortalities (especially in the small cohort), than in increasing growth rates, suggesting that the former largely resulted from reduced incidental anoxia, associated with fewer total numbers of dead animals within growing trays. These results suggest that thinning of cultured oysters may provide a management alternative to partially mitigate losses during the critical period of summer mortalities.

***Histopathology***

Both gross pathology and histopathological evidence suggests an irritant or toxin affecting the epithelial cells of the mantle, causing retraction of that organ and an attempt to "wall off" the oyster's soft tissues from the irritant. We found no evidence to support the contention that juvenile oyster mortality is associated with a newly described protozoan of uncertain affinity (Farley et al. 1992). The coccoid bodies present in many lesions appear to be remnants of degenerated oyster cells, not a protist, and the ciliates seen in some lesions are undoubtedly opportunistic invaders. We conclude that if a microbe is the causative agent of the "disease," it is either not found consistently or in significant numbers in tissues of affected oysters and induces lesions from a distance by production of an exotoxin, or it is easily washed free of the tissues

during specimen preparation for microscopy. It is highly unlikely, however, that a whole population of microbes would be washed from a histological preparation. Furthermore, enough material has been examined at three laboratories (Battelle, HSRL, and VIMS) to make it most unlikely that even a small population of highly virulent microbes would be overlooked.

Juvenile oyster mortality syndrome, including the formation of conchiolin deposits around the contracted oyster tissues, resembles "brown ring disease" of cultured Manila clams, *Tapes philippinarum*, in western Europe (Paillard et al. 1989, Paillard and Maes 1990). The disease was first reported in 1987 when it caused heavy mortalities in cultured clams. Nearly all of the moribund clams exhibited abnormal deposits of organic material on the inner shell. Neither protozoan nor metazoan parasites were detected in histological sections, but a bacterium of the genus *Vibrio* was isolated from diseased clams and caused the brown ring syndrome when injected into healthy clams (Paillard and Maes 1990). Similarly, anomalous conchiolin deposits, generally around the posterior edge of the shell margin, are associated with mortalities of the golden lip pearl oyster, *Pinctada maxima*, in Western Australia. Pass et al. (1987) suggested that *Vibrio harveyi*, isolated from affected pearl oysters, was involved in causing the disease. In both *P. maxima* and *T. philippinarum*, the conchiolin deposits differed somewhat from those of *C. virginica* in that the former were not consolidated into a distinct thin ridge, but rather were spread into a wider band with more irregular borders.

The bacterial etiology of "brown ring disease" in Europe and the fact that histopathological lesions in affected oysters are similar to those found by Dungan and Elston (1988) in association with bacterial destruction of the hinge ligament in juvenile Pacific oysters, *C. gigas*, indicates that a bacterium cannot be ruled out as the cause of juvenile oyster mortality. Whether the bacteria which we found in tissues and in chambers within the anomalous conchiolin deposits are one or several species of opportunistic bacteria or are the causative agent(s) of the mortality remains to be determined. It should be noted that deposition of conchiolin can be affected by other stressors, including handling (e.g. in bay scallops, Palmer 1980) and exposure to anthropogenic contaminants (Hillman unpubl. observations).

In conclusion, the results obtained suggest that oysters may have been affected by a toxin-producing agent (most likely of bacterial or microalgal origin), or by a chemical contaminant which caused mantle retraction and secretion of an abnormal conchiolin layer as a defense mechanism. Death presumably occurred when the muscle became detached from the valve due to conchiolin deposition between the muscle and the shell, and/or degeneration of myoepithelial cells accompanied by bleeding. Mortalities were probably aggravated by entry of secondary invaders into lesions, and by the development of anoxic conditions (in turn aggravated by high summer temperatures) within the trays as oysters began dying. Future work should further investigate site-specificity of mortalities, the influence of rearing temperature, and age/size of affected oysters, the potential role of bacteria, and especially the association of mortalities with the occurrence of blooms of dinoflagellates or other potentially toxic phytoplankton species.

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