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# Daily Growth Increments in the Shells of Larval Sea Scallops (Placopecten magellanicus)

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## ABSTRACT

Larval sea scallops were reared in the laboratory and their shells were examined for growth increments. Major growth increments identified under SEM corresponded to those increments seen under light microscopy. Growth increments were initiated 3 to 4 days after fertilization. Mean counts of growth increments from light micrographs were highly correlated with true age. Age estimates based on growth increment number never differed by more than 3 days from the true age. The daily periodicity of growth increments of larvae was further verified by chemically marking the larval shell with alizarin red.

Photoperiod and feeding frequency had no detectable effect on growth increment count. Increments were laid down on a near daily basis under continual light and when fed every second day.

SPECIAL SESSION ON RECRUITMENT

## INTRODUCTION

Studies of the larval ecology of the sea scallop would be greatly enhanced if it was possible to age scallop larvae. Compared with what is possible now, estimates of such parameters as growth and mortality rates, duration of the pelagic phase, and timing of spawning and metamorphosis would be much more reliable. Although many workers have investigated the nature of skeletal growth increments in adult bivalves (and to a lesser extent juveniles) (Barker 1964, Rheads and Lutz 1980, Rosenberg and Runcorn 1975), few have set out to study the periodicity of growth increments in larval bivalve shells. Millar (1968) reported that in the shells of larval oysters there appeared to be daily and tidal increments which were laid down even in the absence of any environmental cues. Turner and Boyle (1974) observed the growth increments in Teredinid larval shells and suggested that increment counts of known aged larvae would give an indication of their periodicity.

Daily growth increments in otoliths have been used to estimate age since the early 1970's in studies of larval fish ecology (see reviews by Campana and Neilson 1986 and Jones 1986). Recently the approach has been extended to the statoliths of squid (Hurley et al. 1985). Growth increment counts can be used for ageing if the factors affecting the

rate of their formation are understood. The periodicity with which increments are laid down in larval fish otoliths varies between species and can be affected by environmental conditions (see review of otolith microstructure by Campana and Neilson 1985).

In the present contribution we present the results of our investigation of the rate of growth increment formation in larval Placopecten magellanicus. Using both scanning electron microscopy (SEM) and light microscopy, we show that laboratory reared scallop larvae laid down 'major' growth increments with near daily periodicity after day 3.

## METHODS AND MATERIALS

Adult spawners originated from an inshore bed near Yarmouth, Nova Scotia. They were held at Blind Bay, N.S. in off-bottom lantern nets for a period of two to three months prior to spawning. On September 8 1985, male and female scallops were transferred to the Life Sciences Building, Dalhousie University, Halifax N.S. Five individuals of each sex were held separately and induced to spawn by thermal stimulation (Loosanoff and Davis 1963) and injection of seratonin in the adductor muscle (Gibbons and Castagna 1984). Addition of sperm from each of the five males was an additional stimulus for female spawning. The whole process including fertilization was terminated after three hours.

The eggs were maintained in aerated 20 l plastic buckets at a density of 30 eggs per ml of seawater. Culture temperatures of 14 +/- 1 C were maintained by placing the buckets in a controlled temperature water bath. After four days of incubation, the culture water was changed and the larvae were fed for the first time (diet is described below). Most larvae had reached the prodissiconch I stage [see Chanley and Andrews (1971) for a glossary of terms used to describe bivalve larvae] and the density in each of 12 buckets was adjusted to a concentration of approximatedly 2.5 larvae per ml.

The effect of photoperiod and feeding frequency on growth increment number was tested in the following manner. Six buckets were exposed to a 12-hr light: 12-hr dark photoperiod, while the other six buckets were isolated behind black plastic under a 24-hr light regime. The lighting source for both groups was identical (40 W fluorescent bulbs) but the intensity was greater (not measured) for the cultures held under continual light.

Three of the six cultures in each photoperiod treatment were given algal rations daily, while the other three were fed every second day. The daily ration for the larvae in each culture was as follows: 6525 cells per ml of Isochrysis galbana; 3160 cells per ml of Chaetoceros gracilis; and 15315 cells per ml of C. calcitrans. The ration for larvae fed every second day was simply twice the daily ration.

Samples of larvae obtained during the experiment were preserved in 80% ethanol and were usually examined within one month of preservation. Larval length was measured as the longest dimension parallel to the hinge; larval height was the longest dimension perpendicular to the hinge.

Growth increment counts for statistical comparison with actual age were made from light micrographs. The SEM was used to investigate the three dimensional nature of the growth increments.

To provide an additional test for the periodicity of growth increment deposition, a chemical 'time mark' was incorporated into the larval shell. Alizarin red (0.05 mg/l) was added to one of the cultures within the continual light/daily feeding treatment combination on Day 22. This particular culture was maintained until day 30 when the larvae were preserved.

Preparation of the larval shell for ageing - Preserved larvae were transferred to a 0.003 % solution of sodium hypochlorite and allowed to soak for approximately 20 minutes when the valves began to gape. Under a stereomicroscope, the valves were then teased apart using a sharp probe. Etching of the larval valves with different concentrations of hydrochloric acid yielded inconsistent results and was not continued.

A random sample of valves was then pipetted onto a glass slide for viewing with a 32* objective on a compound photomicroscope. Each valve was positioned with the hinge up, and a cover slip added to the preparation.

Right valves were chosen for growth increment counts to ensure that both valves from one larva were not examined. A consistent focal plane for counts was achieved by focussing on the outermost increment near the rim of the valve. At least 10 valves were randomly chosen from each sample and a photographic record of each valve was made using Kodak Technical Pan film (ASA 25).

The growth increment counts were taken from black and white light micrographs printed on high contrast paper. Only those increments which appeared continuous over the whole valve image were counted. Two different readers examined each micrograph and where agreement on growth increment count could not be reached, the micrograph was rejected.

For examination under a Bausch and Lomb scanning electron microscope, the valves were prepared as for light microscopy and pipetted onto a nucleopore filter with a pore size of either 5 or 12 micrometers. The filter and valves were then air dried for a minimum of 12 hours, placed on an SEM stub, and gold plated under vacuum.

## RESULTS

The growth of larval sea scallops under the different experimental conditions was similar until Day 15. Between Days 15 and 28, the growth of the larvae raised in continual light was slower than in the 12 hr light: 12 dark hr photoperiod (Fig. 1), perhaps due to the negative effect of an algal bloom in the continual light cultures.

The first growth increment was visible near the outer region of larval shells sampled on Day 4 under both SEM and light microscopy (Fig.2). Prodissiconch I corresponds to the central region of these valves, and is characterized by shallow, punctate marks (Fig. 2a). Distal to this region is prodissiconch II, where growth increments form. With further shell growth we distinguished 'major' and 'minor' growth increments. Under the SEM the major increments were seen as the most prominent of successive overlapping ridges (Fig. 3). The minor growth increments were less prominent, and also more closely spaced. The finest of these minor increments were less than 1 micrometer apart.

Light micrographs of larval valves showed that the distance between successive growth increments decreased with age (Fig. 4), as expected from the growth curve (Fig. 1). When measured along the height axis the distance between these increments was similar to the distance between major increments identified under SEM (Fig. 5), and to daily increases in height (Fig. 1 ).

Growth increment counts from the light micrographs of larvae from the same treatment combination had coefficients of variation ranging from 3.7 % to 15.3 %. The higher c.v.'s occurred with the youngest animals aged (10 days). When adjusted for age at first increment formation, estimated age correlated well with the true age (Table 1 and Fig. 6). There was no apparent effect of photoperiod or feeding frequency on growth increment number . The difference between estimated age and true age was greatest for 10 day old animals (Table 1).

Alizarin red affected both the behavior and shell structure of the larvae, although the stain was not visible in the shell. The larvae swam much less and the shell was deformed. After removal from the alizarin red, growth increment formation resumed at a daily rate (Fig. 7).

## DISCUSSION

This study provides the first description of growth increments in the shells of larval sea scallops. The major growth increments seen under SEM correspond to those counted from the light micrographs. Estimated age based on mean increment counts from the light micrographs was highly correlated with true age. We conclude that the these major growth increments are formed on a 'near daily' basis, the minor increments on a subdaily basis.

The three dimensional nature of the larval sea scallop valves presents some difficulties in interpreting images from light micrographs. Nevertheless, using a consistent focussing technique, the coefficient of variation of shell increment counts of larvae from the same treatment never exceeded 16 % for young animals, and 9 % in larvae 18 days of age and older. In addition the estimated age based on increment counts of at least 10 larvae never differed by more than 3 for younger animals and by more than 2 for older animals. The better estimate of actual age for older animals may be related to a change in shell curvature, which in turn results in a decrease in the number of subdaily increments in focus.

The deposition of 8 increments in the 8 days following immersion of 22 day old larvae in alizarin red is further evidence that growth increments are laid down on a daily basis. However the shell deformation induced by alizarin red indicates that it is unsuitable as a larval bivalve shell marker. Dey and Bolton (1978) used tetracycline and noted an increase in shell growth rate after marking. Further research into finding an innocuous shell marker is desireable.

Millar (1968) classified growth increments in the larval shells of the European oyster Ostrea edulis, as either fourth or fifth order (6 hr and 24 hr periodicity respectively). However he did not describe his methods for discriminating the two orders of increments. Different orders of increments are also evident in the larval shell of Crassostrea virginica (Carriker and Palmer 1979). In the present study of larval sea scallops, the major increments appear daily, while the minor increments, which we have shown are subdaily, do not appear to occur regularly.

The daily deposition of growth increments under both continual light and feeding every second day tends to support the contention of Millar (1968) that the increments are endogenously laid down. Whether the increments in larval scallop shells are actually endogenous would be difficult to prove. In any case counts of growth increments on the shells of field caught larvae should provide a reliable estimate of age if it can be shown that other factors such as temperature and starvation do not affect the rate of increment formation. This work is presently ongoing.

## ACKNOWLEDGEMENTS

We wish to acknowledge the dedicated effort of R. Hartt. in rearing the scallop larvae and preparing the shells for ageing. C. Mason provided valuable assistance with the SEM. The cooperation of N. Balch, manager of the Dalhousie Aquatron Laboratory is most appreciated. The work was encouraged by M. Sinclair.

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Table 1. Estimated larval age from light micrographs compared to actual age. Counts of growth increments have been adjusted for the day of first increment formation (4 days after fertilization) by the addition of 3. In each cell is the mean, the number of specimens examined, and the coefficent of variation.

| | Actual age (days) | | | |
|---|---|---|---|---|
| | 10 | 18 | 24 | 28 |
| Treatment | | | | |
| 12:12 hr photoperiod; daily feeding | 10.90<br>39<br>15.3% | 18.80<br>11<br>6.2% | 25.43<br>40<br>8.3% | 27.80<br>10<br>3.7% |
| 12:12 hr photoperiod; bidaily feeding | 11.60<br>39<br>13.0% | 18.70<br>11<br>6.6% | 25.00<br>38<br>4.9% | 28.00<br>10<br>6.1% |
| continual light; daily feeding | 10.30<br>40<br>13.2% | 19.73<br>11<br>7.9% | 25.05<br>40<br>5.3% | 27.90<br>10<br>4.3% |
| continual light; bidaily feeding | 11.20<br>40<br>15.1% | 18.64<br>11<br>10.5% | 25.10<br>40<br>5.8% | 27.90<br>10<br>5.2% |

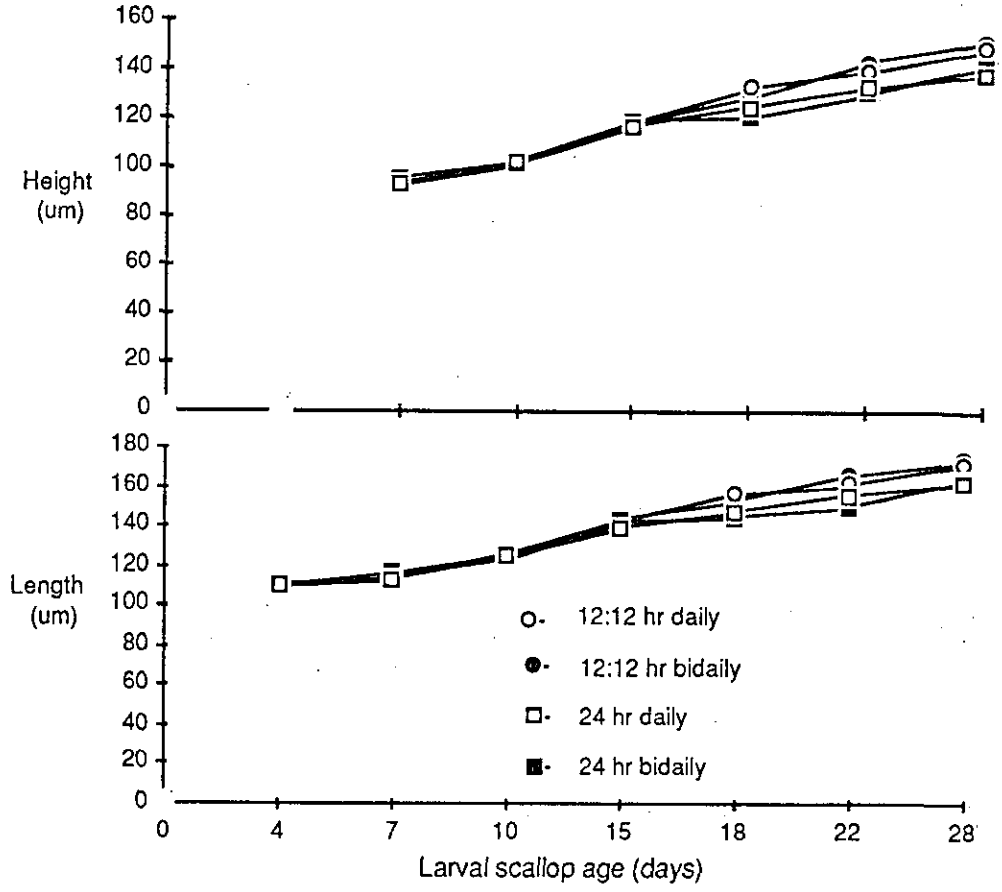
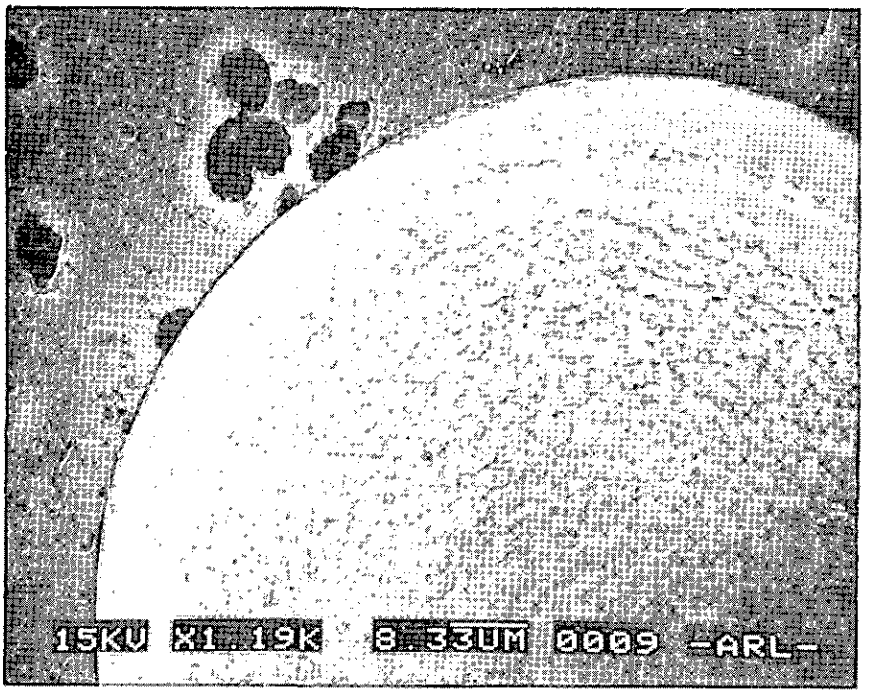



Figure 1. Growth of larval scallops reared under different light conditions and fed either daily or every second day.



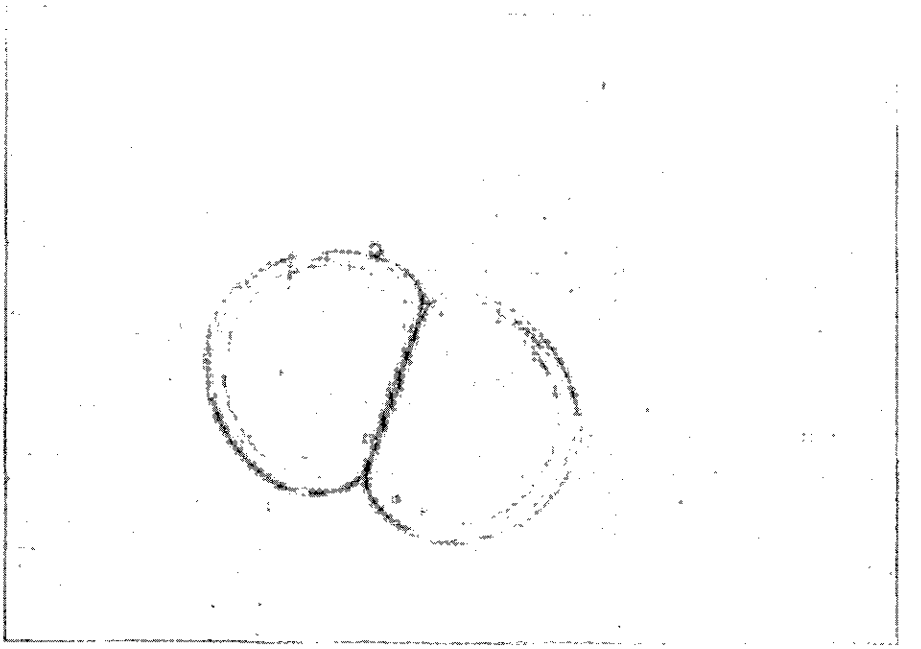
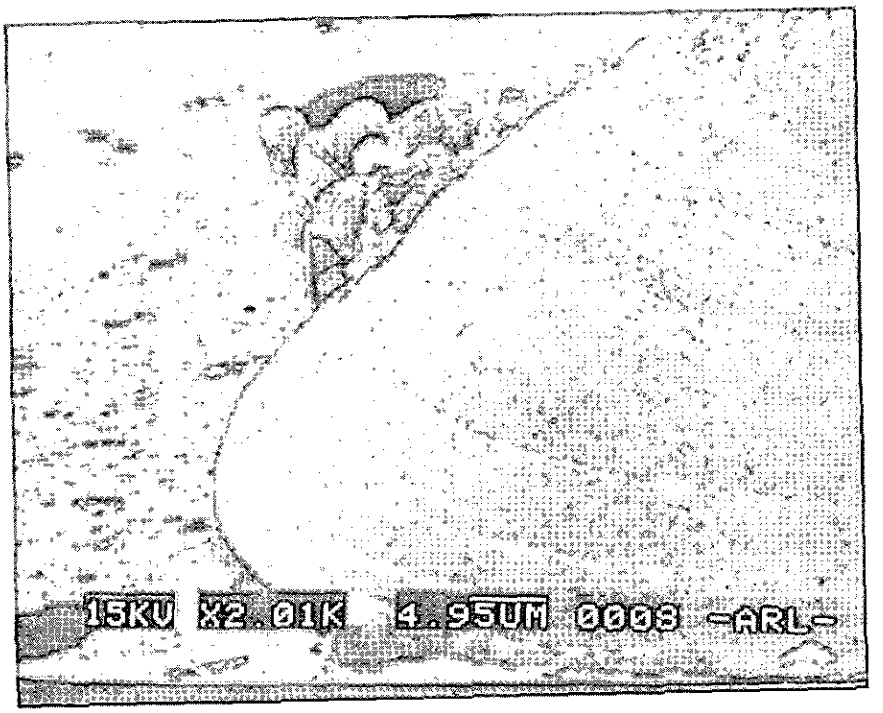



Figure 2. Shell valves from four day old larvae showing first growth increment. (a) SEM micrograph of exterior surface - outer edge. (b) Light micrograph of valves still attached via hinge. Interior view. Magnification 450*.



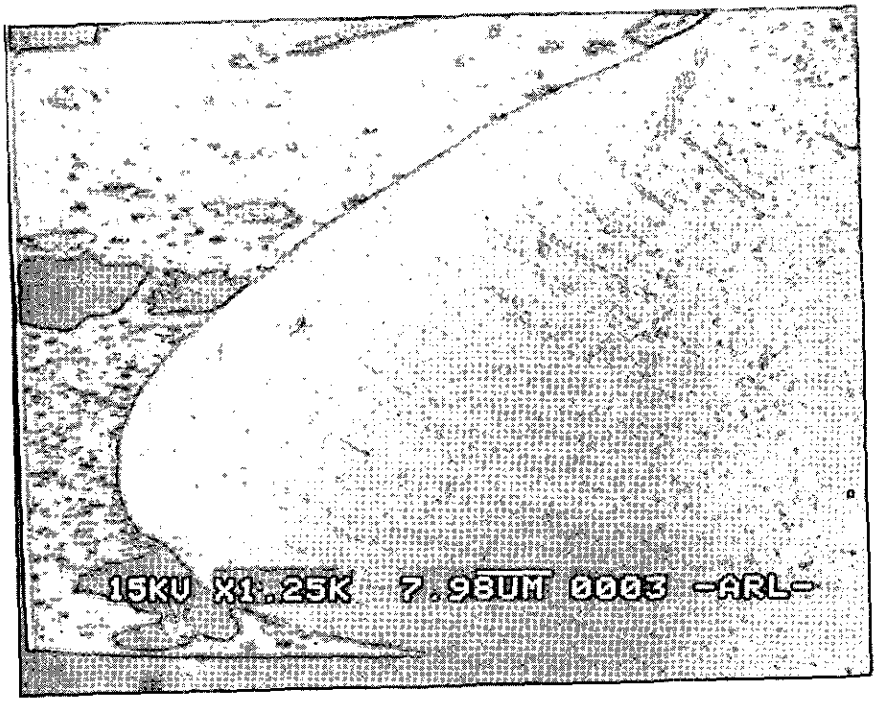




Figure 3. Scanning electron micrographs of the exterior surface of larval scallop valves. (a) 10 day old valve. Cultured under 12-hr light: 12-hr dark photoperiod and fed daily. (b) 24 day old valve. Cultured under continual light with feeding every second day.

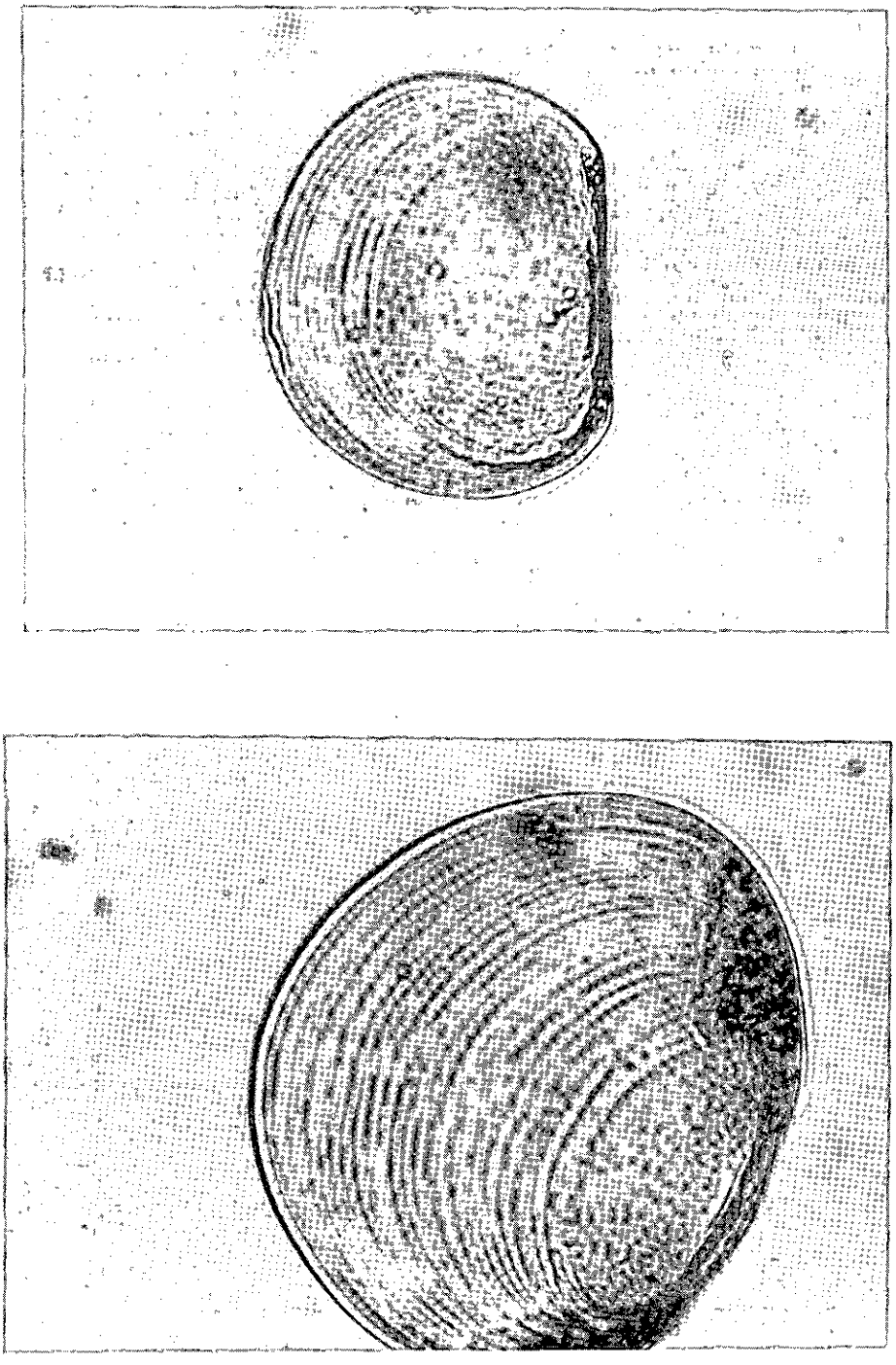


Figure 4. Light micrographs of larval scallop valves. (a) 10 day old valve, interior view. Cultured under continual light with daily feeding. (b) 24 day old valve. Cultured under 12-hr light: 12-hr dark photoperiod with daily feeding. Magnification of (a) and (b) 450*.

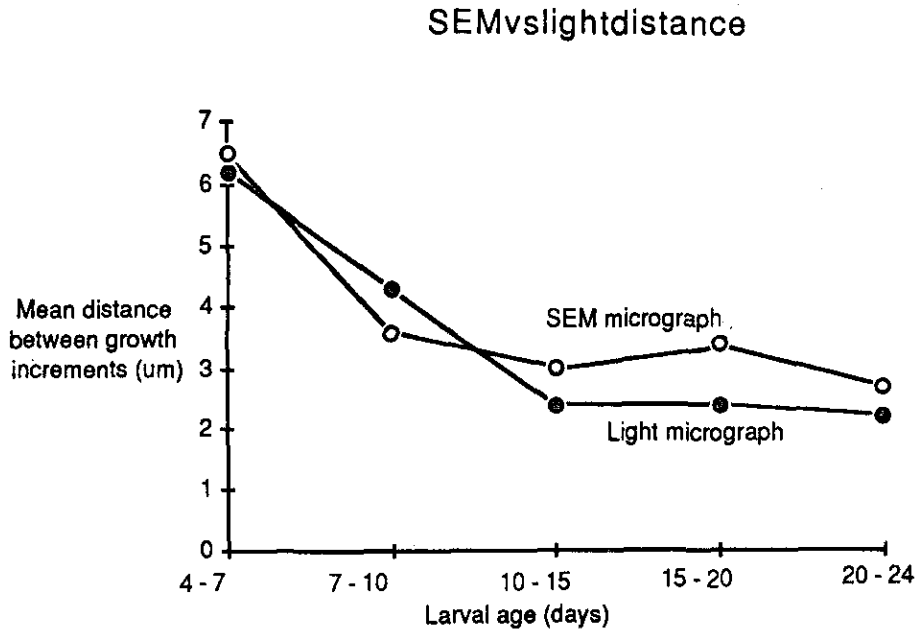



Figure 5. Mean distance between daily growth increments measured along height axis: SEM versus light microscopy. Larvae measured were of equal size, and 24 days old. Cultured under 12-hr light: 12-hr dark photoperiod, feeding every second day.

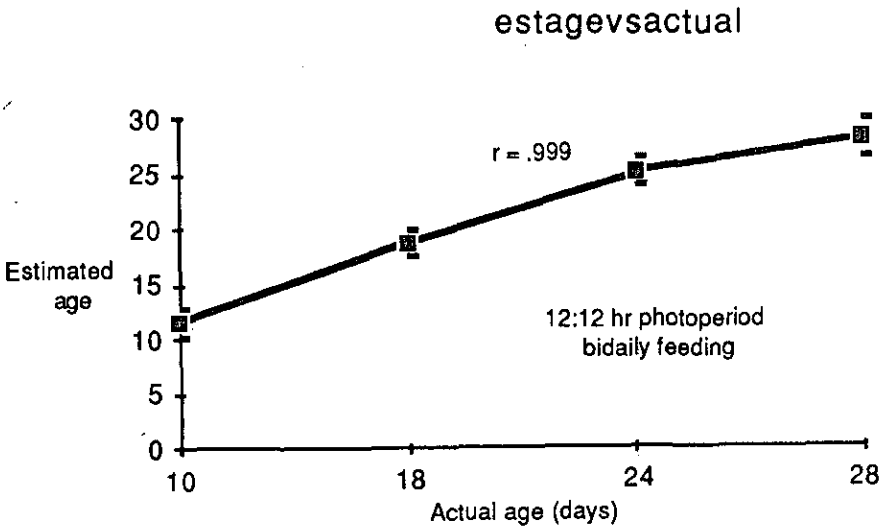
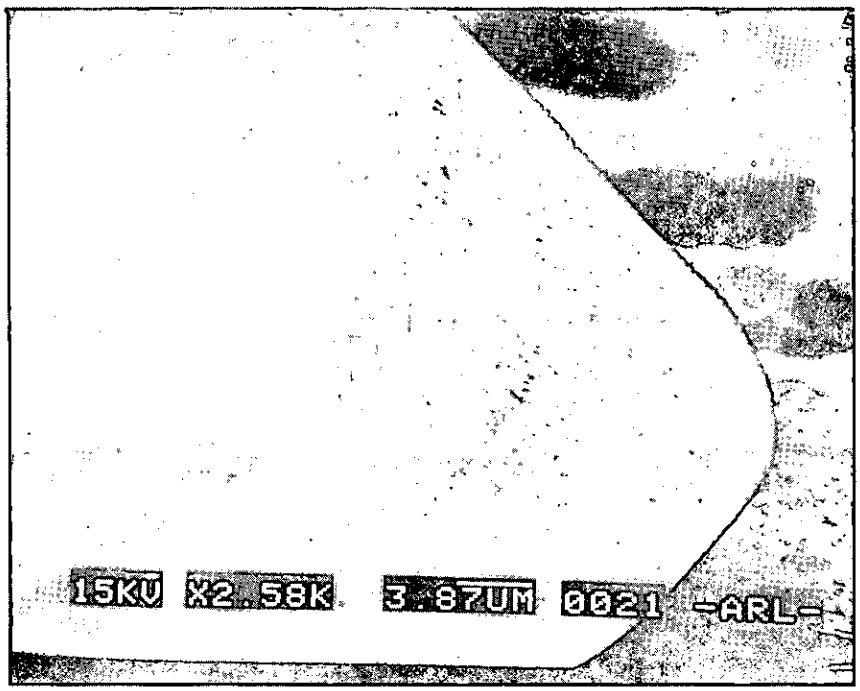



Figure 6. Age based on increment count plus 3 (to account for age at which first increment formed) versus actual age. Each point is the mean of at least 10 counts. Bars are one standard deviation.



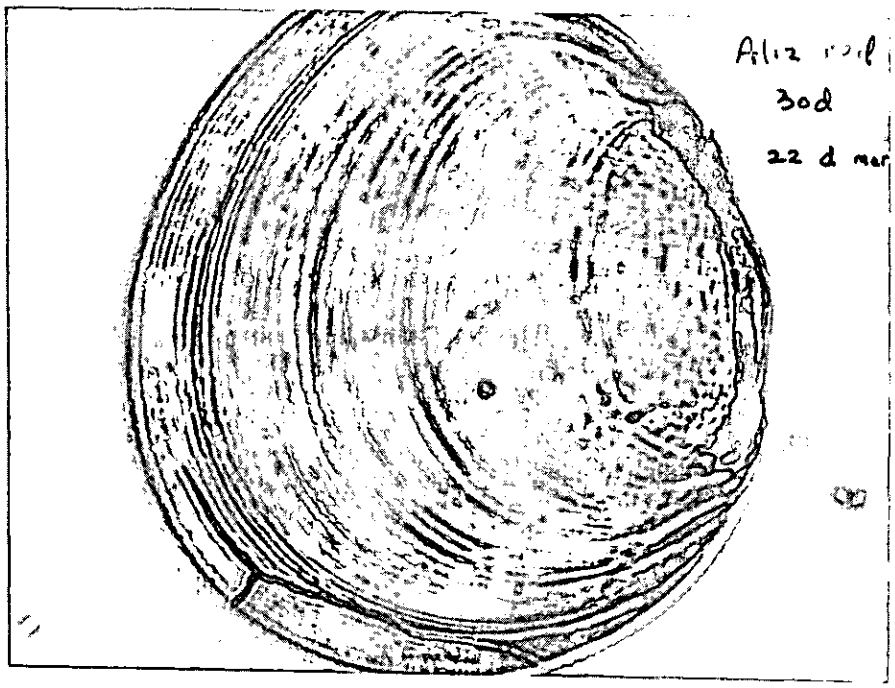




Figure 7. 30 day old valves which were marked by the addition of alizarin red when 22 days old. (a) SEM micrograph (b) light micrograph.