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Title: GROWTH OF A SEA URCHIN, ALLOCENTROTUS FRAGILIS, AT DIFFERENT DEPTHS OFF THE OREGON COAST

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Allocentrotus fragilis (Jackson) was obtained from six stations at depths of 100 to 1260 m on the continental shelf and upper slope off Newport, Oregon.

Ages and growth rates of A. fragilis were determined by two methods: 1) A procedure was developed to make growth zones of the skeletal test plates visible. Alternating light and dark growth zones were found to be formed semi-annually. The total number of growth zones was used to indicate the urchin's age. 2) Age and growth rate values were also determined from analyses of size-frequency distributions of trawl collections from 200 m. Collections from other depths were not adequate for size-frequency analyses.

Gonad indices of A. fragilis from 200 m were used to determine spawning periodicity and frequency. A semi-annual frequency was found, with spawning occurring in early spring and early autumn. No A. fragilis specimens collected below 400 m were reproductively

mature.

The growth curve of A. fragilis from 200 m, which was plotted from the mean test diameter of age groups defined by test plate growth zones, shows a good least-squares fit with von Bertalanffy's growth equation. Growth rates were similar for A. fragilis from 100-600 m, but decreased for specimens from 800 and 1260 m. The maximum test diameter decreased with increasing depth below 200 m.

Variation of magnesium content of the calcareous skeletal plates was due largely to variation with age. Little skeletal Mg variation was found at different depths for specimens of the same age. Greater Mg content differences occurred between young and old specimens from the same depth, and between young and old test plates of the same individual.

The effects of several environmental factors on the growth rate and maximum size of A. fragilis are discussed. Of these factors, food availability, water temperature, and oxygen tension form gradients with depth or distance offshore; and were considered to be important in affecting the growth of A. fragilis.

Growth of a Sea Urchin, Allocentrotus fragilis, at Different Depths off the Oregon Coast

by

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GROWTH OF A SEA URCHIN, ALLOCENTROTUS FRAGILIS, AT DIFFERENT DEPTHS OFF THE OREGON COAST

INTRODUCTION

The pink sea urchin Allocentrotus fragilis (Jackson) inhabits the continental shelf and upper slope of the west coast of North America from Baja, California to Vancouver Island (Mortensen, 1943). The bathymetric range of this species was reported by Mortensen (1943) to extend from 50 to 1150 m. McCauley and Carey (1967) have discussed and summarized the distribution of this species. In Monterey Bay, Boolootian et al. (1959) found young A. fragilis inhabiting rock burrows as does Strongylocentrotus purpuratus (Stimpson), a closely related intertidal urchin. The physiology, reproduction, and ecology of this population were studied (Moore, 1959; Boolootian et al., 1959; and Giese, 1961), but no measurements were made of age or growth rate.

Allocentrotus fragilis has often been collected in large numbers off Oregon where it is found on unconsolidated sediments, ranging from sand to clayey silts (McCauley and Carey, 1967). Stander (1970) reported A. fragilis as the dominant benthic organism, in terms of biomass, at 200 m off Newport, Oregon. Yet no information on the age or growth rate of A. fragilis or other deep-water echinoids is available. This has generally been due to the inaccessibility of the

study population, and to the excessive costs of repeated deep-water sampling. However, a variety of benthic sampling programs by Oregon State University Department of Oceanography have yielded large numbers of A. fragilis for study.

The goals of this study were to determine the ages and the growth rates of A. fragilis from various depths within its bathymetric range. Such information is necessary and basic to understanding the ecology of the species.

An important requisite was the development of an accurate method of age determination for A. fragilis. Ages and growth rates of sea urchins have been determined by one or a combination of the following methods: 1) tagging and observation of selected individuals in their natural habitat, 2) observation of urchins maintained in the laboratory, 3) size-frequency distribution analyses, or 4) analyses of cyclic growth indicators in the spines and test plates.

Methods of individual tagging, such as that used by Ebert (1965), are an effective way of following the growth of individual urchins, but are restricted to the intertidal area where urchins may be collected, tagged, and replaced for later recovery.

Growth rates may also be determined from laboratory growth studies. This method is also normally restricted to shallow water urchins, because deep water species such as A. fragilis quite often do not survive the rigors of collection or do not survive long in the

laboratory if collected alive (Boolootian et al., 1959). A few A. fragilis from 200 m off Oregon survived in aquaria for a maximum of only five months (Giles, personal communication); a time insufficient to obtain adequate growth measurements.

Size-frequency distributions have been used successfully by Fuji (1967) and Ebert (1968) to establish the ages and growth rates of two intertidal species of Strongylocentrotus. Periodic large samples representative of a population are required.

Cyclic patterns observed in the hard parts of various marine invertebrates have been related to periodic variations in the structure or pigmentation during growth of the hard part. These include daily growth lines of pelycepod shells (Clark, 1968; House and Farrow, 1968) and seasonal pigment bands in the shells of abalone (Olsen, 1968). Many echinoids also exhibit cyclic patterns in their test plates and spines which are superficially similar to the annual growth rings found in woody plants.

The literature on the use of echinoid spine rings as age indicators has been summarized by Ebert (1967b). He found a general correlation between the test diameter, a measure of relative age, and the number of rings in the spines of S. purpuratus. He attributed the formation of new spine rings to regeneration of the spine tip, and not to a periodic growth cycle. Ebert felt that the correlation between the number of spine rings and the test diameter was fortuitous,

as large urchins would incur more spine breakage simply because they were older. Weber (1969a) studied the spine rings of Heterocentrotus trigonarius (Lamarck) and found a very good correlation between the test diameter and the maximum number of spine rings found on that individual. As the frequency of spine ring formation was not known, Weber was uncertain about the interval of time represented by one spine ring. A lunar periodicity was suggested.

The cyclic growth zones observed in the test plates of echinoids have received less notice as age indicators, yet interpretation is theoretically much simpler than for spine rings. Breakage and loss of plates do not normally occur, whereas spine loss and damage is common (Ebert, 1967a; Weber, 1969a). The earliest formed test plates are always associated with the urchin for its entire non-larval life and therefore should more accurately represent the age of the urchin. Also some echinoids, including A. fragilis, have spines which are too small to be amenable to the ring count method.

Deutler (1926) ground the test plates of a variety of echinoid species to make the growth zones visible. Using a similar method with the genital plates of Echinus esculentus Linneus, Moore (1935) found good agreement between the number of growth zones and the age of the urchins as determined from size-frequency distributions. He noted a zone with darker pigmentation was added to each test plate during the summer, and a lighter non-pigmented zone was added

during the winter.

A much less tedious method of making the growth zones visible by clearing the test plates with an organic solvent was described by Jensen (1969). For the present study, a modification of this method was used in conjunction with size-frequency distribution analyses to determine the age and growth rate of A. fragilis.

METHODS AND MATERIALS

Allocentrotus fragilis specimens used in this study were collected by the Oregon State University Department of Oceanography as part of regular sampling programs. The Oregon State University Department of Oceanography research vessels Acona, Yaquina, and Cayuse were used to obtain these collections.

Samples of A. fragilis from 100, 200, 400, 600, 800, and 1260 m were obtained along a line extending west from Newport, Oregon near latitude 44° 39'. Only samples from the stations along this sampling line were used, although A. fragilis has been collected in large numbers from stations to the north and south.

Allocentrotus fragilis was collected with a non-quantitative otter trawl and a semi-quantitative three-meter wide beam trawl. The beam trawl was equipped with two odometer wheels to measure the distance travelled on the bottom. Population density at 200 m was estimated from a beam trawl collection. Both trawl types were fitted with a 3.5 cm stretched mesh net and a 1.2 cm stretched mesh net liner and were assumed to have similar sampling characteristics.

Many of the 200 m trawl collections yielded large numbers of A. fragilis. These were subsampled at sea, with most of the urchins thrown overboard. Some bias of subsampling probably occurred

as the smaller, more fragile urchins were more easily broken during trawling. These urchins were probably often rejected in preference to larger, whole animals. In more recent collections (during 1969 and 1970) the subsampled urchins were counted, and their test diameters were recorded before being discarded.

The urchins were preserved aboard ship in 10% formalin in sea water. This was replaced in the laboratory with 70% isopropanol. In a few instances the urchins were frozen aboard ship, thus eliminating any contact with preservatives.

Sediment samples and bottom water samples were obtained at each station using a modified Smith-McIntyre sediment grab equipped with a water sampler and reversing thermometers (Carey and Paul, 1968). Bottom water temperature, salinity, and dissolved oxygen values were determined.

Those urchins returned to the laboratory were scrubbed with a stiff brush to remove the spines. The test diameter, from an ambulacrum to the opposite interambulacrum, was measured to the nearest millimeter with knife-edged vernier calipers.

The thin, fragile tests of A. fragilis were opened with a fine-toothed jeweler's saw blade attached to a 60-cycle electric vibrator (Brownell, 1970). The gonads were removed, preserved in 70% isopropanol, and weighed to the nearest 0.01 gm after a standardized one minute blotting time. A gonad index was computed from the

following formula:

$$\text{Gonad Index} = \frac{\text{gonad wet weight}}{\text{test diameter}} \times 100 .$$

The gonad index values ranged from less than 0.1 for immature specimens up to 3.3 for mature individuals.

The entire alimentary tract was dissected out, the contents removed and examined under a 6x dissecting microscope. No attempt was made to separate foregut contents from hindgut contents. The percent of organic matter contributing to the dry weight of the gut contents was determined for each collection by drying to constant weight at 65° C, then ashing in a muffle furnace at 425° C for 48 hours to remove organic material.

Jensen (1969) described a simple method for demonstrating growth zones of sea urchin test plates by heating the plates over an alcohol flame, then wetting with xylene. The method used by Jensen on Strongylocentrotus droebachiensis (D. F. Müller) and other urchins with relatively thick test plates was initially used on A. fragilis, but was found to be unsuitable for making visible the growth zones of the much thinner test plates of this species.

The following procedure for treating the test plates of A. fragilis evolved from the method used by Jensen (1969). The two halves of the emptied test were rinsed, then placed in 25% household bleach (e.g. "Chlorox") until most of the organic material was removed. The test

halves were removed from the bleach solution before disarticulation of the test plates occurred. One interambulacral column and the adjacent ambulacral column of test plates extending from the peristome to the periproct were separated from the remainder of a test half. The plates were arranged in order, flat, on a 1 x 3 inch microscope slide with the inner side of the test plates up. The test plates, arranged on the slides, were then heated on a hot plate at 85° C for three hours to remove all moisture. After cooling, the plates were wetted with xylene, then saturated with "Permount" and allowed to dry. Test plate growth zones could then be counted under a 6x dissecting microscope.

Two modifications of Jensen's method (1969) were made to make visible the growth zones in the test plates of A. fragilis. An electric, solid top, hot plate rather than an open alcohol flame was used to heat the test plates. This provided a much more even heating effect with less resultant discoloration of the test plates. "Permount" was substituted for xylene and provided the same visible contrast of growth zones as did xylene (which Jensen used) but did not evaporate or need replacing after each examination. Also "Permount," when dry, became an effective bonding medium between the test plates and the glass slide.

The $MgCO_3$ and $CaCO_3$ contents of the test plates were determined in the following manner. A complete ambulacral and the adjacent interambulacral column of each specimen was dissected after

cleaning with 25% household bleach. For smaller urchins, more test material was utilized, but equal numbers of ambulacral and interambulacral columns were always used. The test plates were dried, crushed to a coarse powder, then weighed. The powdered test material was dissolved in 5% HCl, then diluted appropriately with distilled deionized water for analysis. The Mg and Ca concentrations were determined using a Perkin-Elmer model 303 Atomic Absorption Spectrophotometer (AAS). Methods of analysis were taken from a manual by Perkin-Elmer Corporation (1968). Magnesium standard solutions were prepared from analytical grade $Mg(NO_3)_2 \cdot 6H_2O$, and Ca standard solutions from analytical grade $CaCO_3$. A 1% Li_2O_3 solution was used to supress cationic interference for Ca determinations.

The method described by Tomlinson and Abramson (1961) was used to obtain a least-squares fit of growth data to the von Bertalanffy growth equation. An Olivetti Underwood Programma model 101 was used for the remaining statistical analyses. Analysis programs were taken from an Olivetti Underwood manual by Williams (1968).

RESULTS AND DISCUSSION

Sampling Results

Of the six stations sampled, A. fragilis was taken consistently only at the 200 m station (Table 1). The values given in the last row are not directly comparable between stations, as the duration of trawling time was increased for the deeper stations. They are given only as a rough indication of A. fragilis abundance at each station.

Table 1. Summary of A. fragilis collections at latitude 44°39' since April, 1963.

Station Depth:	100 m	200 m	400 m	600 m	800 m	1260 m
Number of trawls	10	38	4	6	26	6
Trawls with A. fragilis	1	38	3	3	4	1
Percent with A. fragilis	10	100	75	50	15	17
Maximum number A. fragilis/trawl	16	750	35	93	89	5

A beam trawl collection made on 15 March, 1970 at the 200 m station yielded 687 A. fragilis during a 10 minute trawl. Approximately 3200 m^2 of bottom area were sampled. The average A. fragilis density thus calculated was 0.2 urchins x m^{-2}. This was less than the 0.5 A. fragilis x m^{-2} reported by McCauley and Carey (1967) for the same area. Only otter trawl samples were obtained at the other stations. McCauley and Carey (1967) have shown, using a Benthos Time-Depth Recorder, that the otter trawl may be off the bottom as much as 75% of the time, making estimates of the area

sampled extremely difficult and unreliable. Even so, it can be seen from the data provided in Table 1 that A. fragilis is much more abundant at the 200 m station than at the other stations. The lowest density probably occurs at the deepest station (1260 m) with intermediate densities at depths between 200 m and 1260 m.

The smallest A. fragilis test diameter taken in any of the trawls was 8 mm. This was assumed to be the minimum size catch capability of either the beam or the otter trawl nets. The number of A. fragilis with test diameters smaller than the stretched size of the net liner (12 mm) was probably not representative of the sampled population due to some loss of these small urchins through the net mesh.

The collection of five A. fragilis from 1260 m (latitude 44° 36', longitude 125° 02') has extended the bathymetric range of this species 110 m deeper than the 1150 m previously reported by Mortensen (1943).

Age and Growth Rate

The age and growth rate of A. fragilis were determined by two methods. Visible growth zones in test plates of 5-30 specimens from each trawl collection were counted and related to age. Size-frequency distributions of successive trawl samples from the 200 m station were also studied in conjunction with the analysis of A. fragilis

reproductive periods.

Test Plate Growth Zones

Jensen (1969) found for the North Atlantic intertidal urchin, Strongylocentrotus droebachiensis, that one pair of test plate growth zones was added each year; a narrow, dark zone during the summer, and a light, wider zone during the winter. The darker growth zones were thought to contain a higher content of organic pigments (Deutler, 1926; Jensen, 1969) which apparently discolored and became more visible when heated.

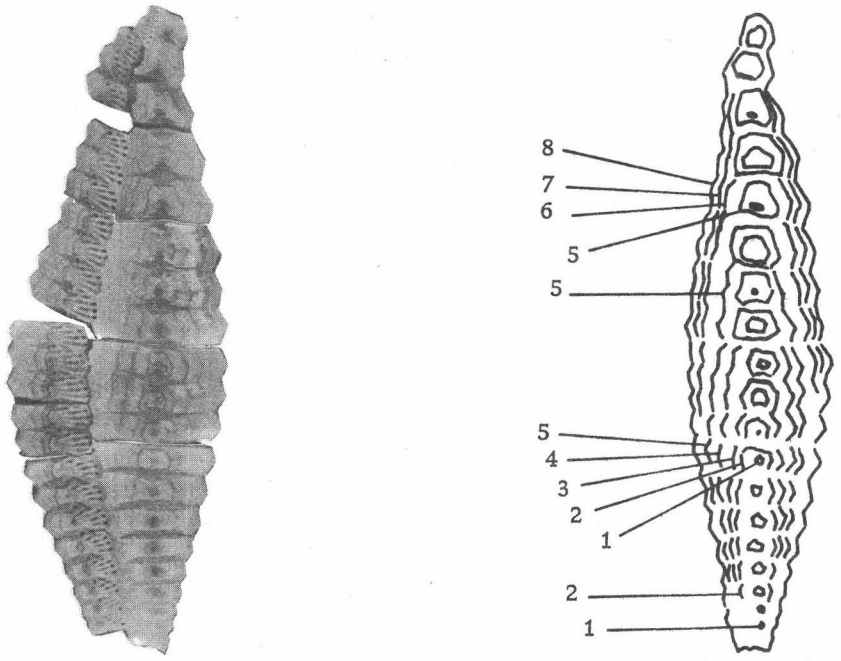
Zoeke (1952) examined fossil tests of Hemicidaris sp. and observed alternating bands of coarse and fine fenestrae within the test plates. These structural differences may have been the result of differential preservation of the calcite due to variations in organic content. Moore (1935) found the darker summer zones of Echinus esculentus to be pigmented and also harder than the light winter zones. Similarly, the concentric dark bands of the spines of S. purpuratus are composed of fine calcite crystals with echinochrome pigments. The alternating light zones have larger crystals and little or no pigment (Ebert, 1967b). Thus, the alternating growth zones in echinoid test plates appear at least to differ in the amount of contained pigment or other organic material. Structural differences of the calcite may also be associated with growth zones. Both Moore

(1935) and Jensen (1969) concluded that the maximum number of dark (or light) growth zones in the test plates represented the urchin's age in years.

The following description of echinoid skeletal growth and growth zone addition within the test plates is based on Jensen's study of S. droebachiensis (1969) and the present study of A. fragilis. Reference to Figure 1 may be helpful. The maximum number of growth zones in A. fragilis is not exhibited in any one plate of an interambulacral column. The plates near the peristome are the first formed and therefore the oldest. As the urchin grows, the width of the existing plates increases, and new plates are formed in the apical region. As more plates are formed they migrate from the apical region toward the ambitus, the region of widest test diameter. As the plates approach the ambitus they increase greatly in width, but little vertical growth occurs. It is in the wide lateral areas of the test plates that the growth zones are best seen and counted.

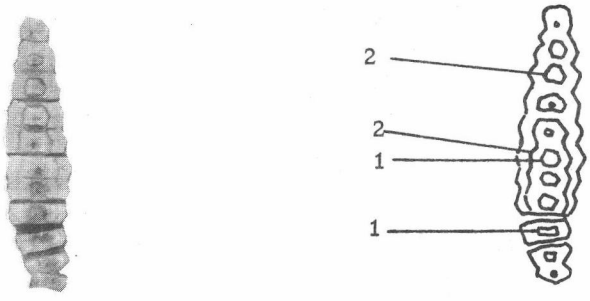
As the test diameter continues to increase during growth, the older plates migrate past the wide ambitus region toward the more narrow peristome. These older plates cease lateral growth as they pass the ambitus because the test area in which they are now located is no longer increasing in size.

Cessation of lateral growth halts the formation of growth zones in these plates; but growth zones continue to be formed in the growing



A A'

X 1.7



B B'

Figure 1. Photographs and tracings of interambulacral columns of two A. fragilis specimens from 200 m. A. from a specimen 53 mm in test diameter and B. from a specimen 17 mm in test diameter. The dark growth zones are numbered in A' and B' according to the order in which they were formed.

plates above the ambitus. The overall effect of this type of plate growth causes the oldest plates near the peristome to exhibit only the earlier formed growth zones but not the more recent zones. The younger plates, near the apical area, do not exhibit the early growth zones because these plates did not exist when the early zones were being formed. These younger plates show only the more recent growth zones, some of which are not exhibited by the older plates.

Therefore, growth zone counting required beginning with the oldest plates near the peristome, counting growth rings laterally, moving up the corresponding growth zone to a younger plate, and counting laterally again. This process was repeated until all the growth zones within an interambulacral series had been counted. Figure 1 illustrates the zones prepared for counting.

The maximum number of dark growth zones were determined for one interambulacral plate series of each urchin examined. The least number of dark growth zones found was one, in specimens 8-9 mm in diameter; the greatest number was 15 in a specimen 80 mm in diameter. These specimens were collected from the 200 m station.

The test diameters were plotted as a function of the number of dark zones of the specimen (Figures 2, 3 and 4). A first approximation to the actual growth curve of A. fragilis from 200 m was obtained by drawing a visual best fit curve through the data points

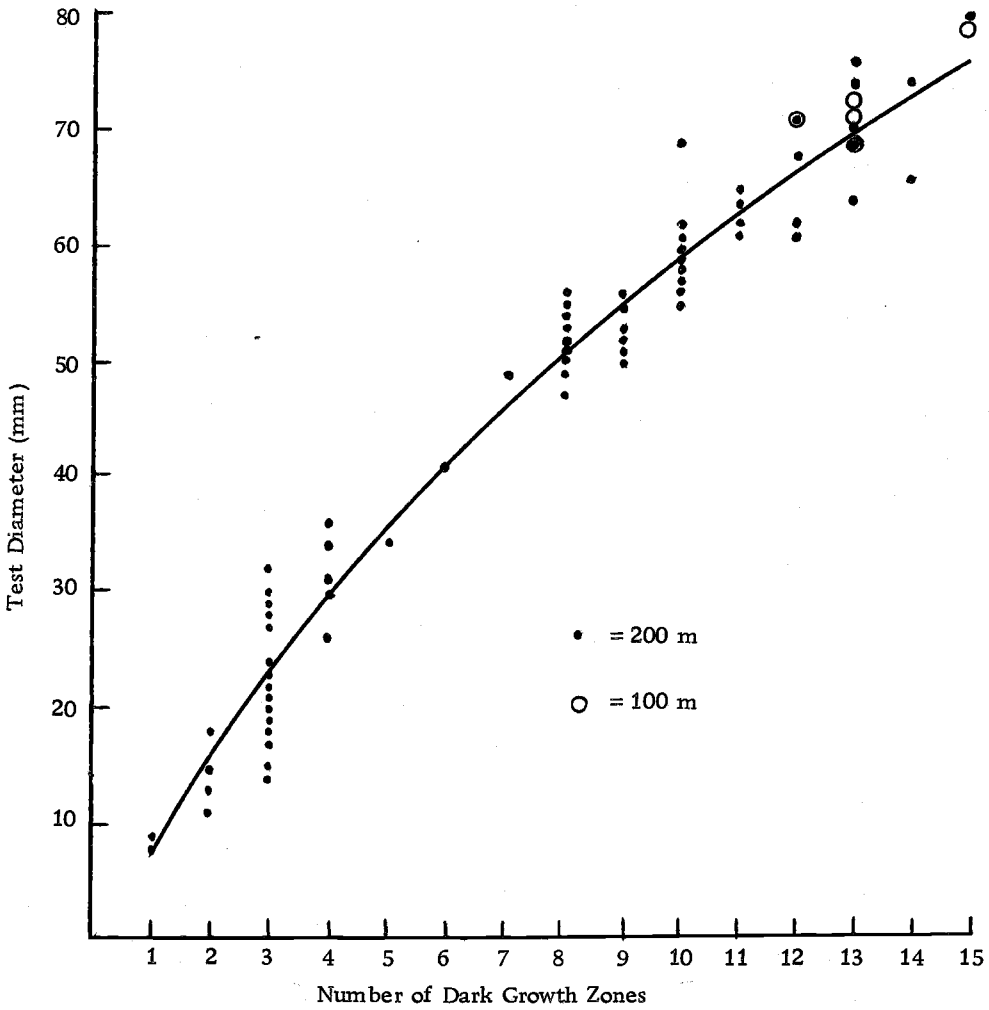


Figure 2. Correlation of test diameter and number of dark growth zones of A. fragilis from 100 and 200 m.

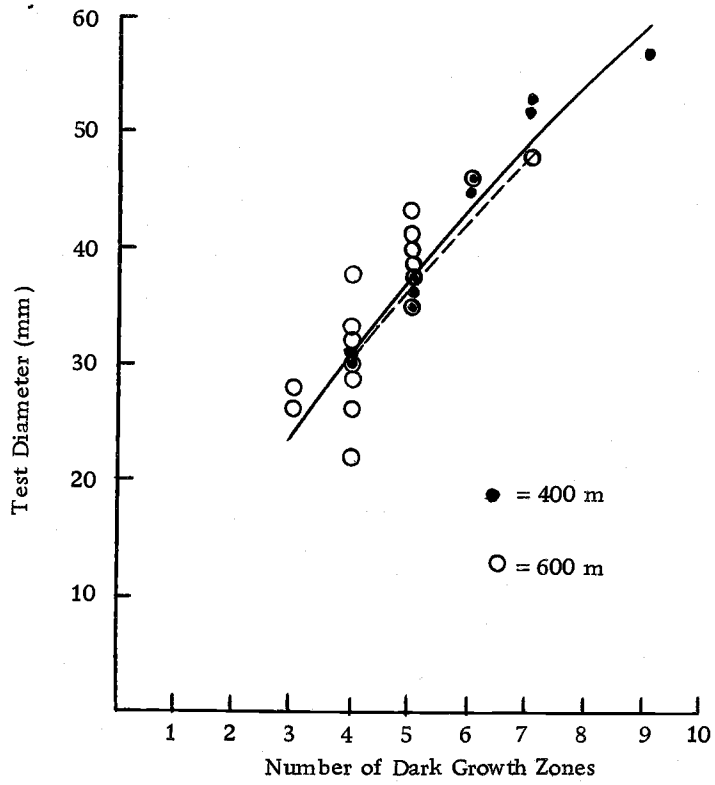


Figure 3. Correlation of test diameter and number of dark growth zones of A. fragilis from 400 and 600 m.

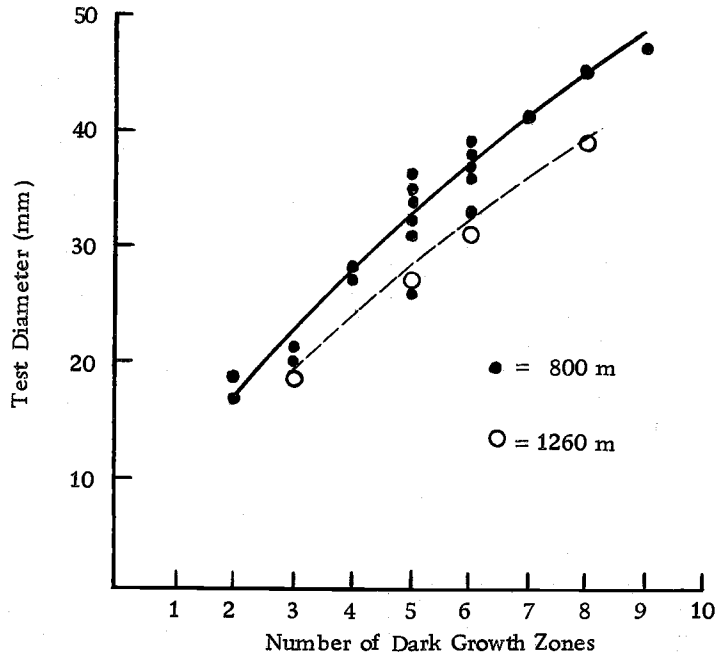


Figure 4. Correlation of test diameter and number of dark growth zones of A. fragilis from 800 and 1260 m.

(Figure 2). Similar growth curves for the other stations, except 100 m, were constructed. It was apparent that specimens from the deeper stations exhibited smaller maximum test diameters and slower growth rates. Urchins from 200 m have approximate growth rates of 9 mm per growth zone period for 15 mm urchins, and 7 mm per growth zone period for 30 mm urchins (Figure 2). The slope of the growth curve decreased to about 3 mm per growth zone period for the largest and presumably oldest urchins.

Size-frequency Distribution Analysis

The usefulness and accuracy of size-frequency distributions as indicators of age depends on the following factors: 1) size-frequency peaks of successive age groups must be discernible; 2) all animals within an age class must have similar growth responses, thus eliminating polymodality within an age class; 3) the population sample should include representative numbers from all size classes present in the population; and 4) spawning must occur periodically with a spawning duration short in relation to the length of the reproductive cycle. Growth rates may be determined from age and size measurements, or by determining the mean size increase of an age class over a period of time.

Trawl collections of A. fragilis from stations other than 200 m were neither numerous nor periodic. The size-frequency distributions

of all available A. fragilis collections from the 100, 400, 600, 800, and 1260 m stations are shown in Figure 5. Age and growth rate determinations from size-frequency distributions were not attempted for these stations due to small numbers of individuals per trawl and to the lack of successive periodic collections at any one station.

The test diameters of 2487 A. fragilis specimens from the 200 m station were measured. The specimens were from 11 trawl collections taken between October 1968 and March 1970. The size-frequency distributions for 7 of the 11 trawl collections are shown in Figure 6. The four additional samples taken during that period had only large specimens (greater than 50 mm) and were not included. The October 1968, September 1969, and November 1969 distributions represent complete trawl collections. The remaining trawls were subsampled at sea without obtaining test diameters of those urchins discarded. Biased subsampling of the January 1969, April 1969, July 1969, and March 1970 trawls may partially account for the noticeable lack of small urchins in these collections.

The trawl collections taken in September and November 1969 have two well-defined size classes each with the means of each size class centered 10-11 mm apart. By assuming that each peak represented a separate age class and that the two November peaks were derived from the slightly smaller September peaks, two independent measures of A. fragilis growth rate were determined. The increase

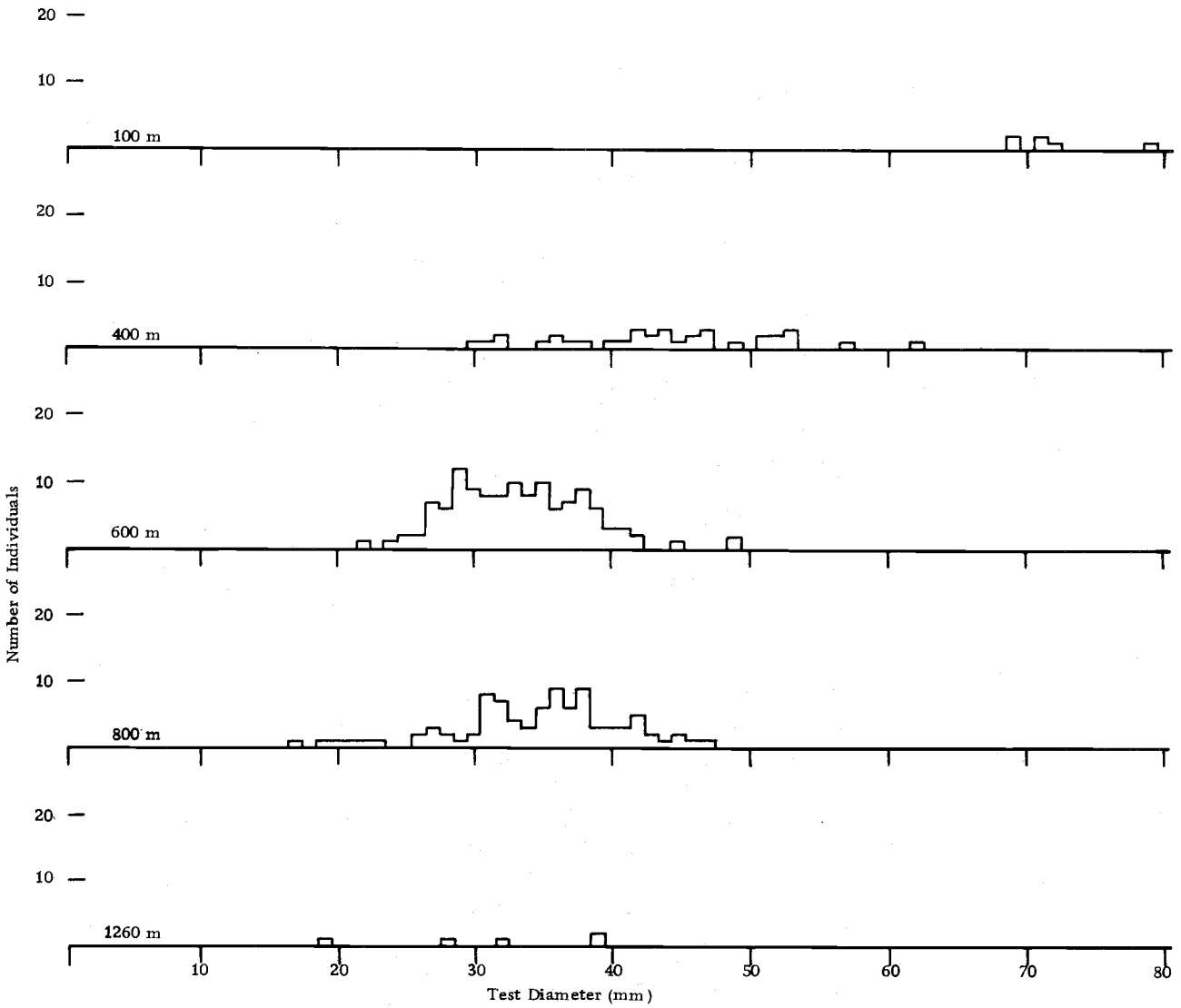


Figure 5. Size-frequency distributions of A. fragilis from 100, 400, 600, 800, and 1260 m.

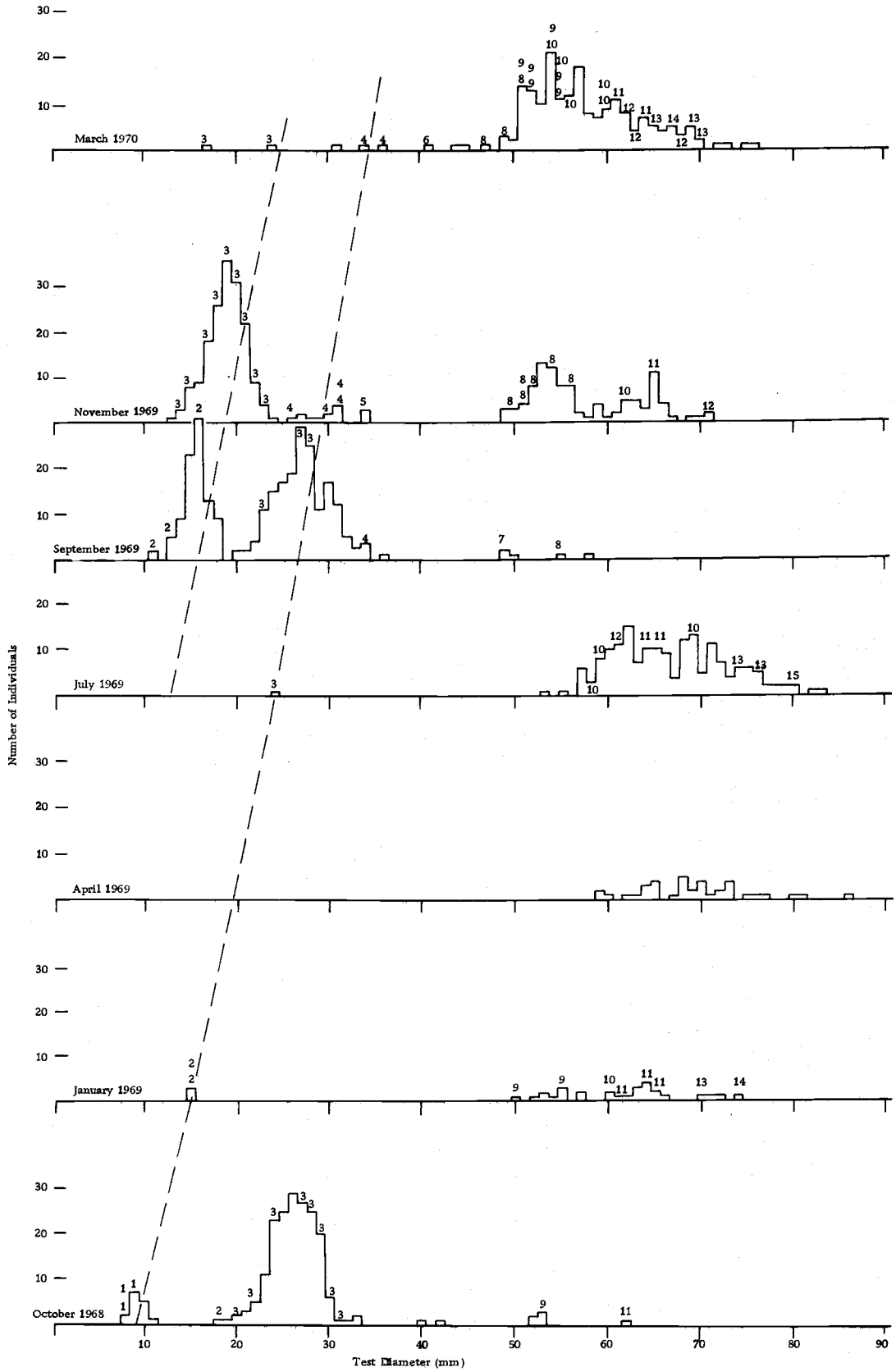


Figure 6. Time series of A. fragilis size-frequency distributions from 200 m. Numbers above each size increment indicate the number of dark growth zones found in specimens of that size. Dotted lines intersect the means of well defined size classes indicating assumed size increase with time.

in the mean test diameter of each size class during the two month interval between the September and November collections was 3.2 mm for the smaller peak and 2.6 mm for the larger peak. This was equivalent to approximately 20 and 16 mm of annual test diameter increase for the two size classes. No attempts were made to resolve statistically the modal classes of the larger urchins. Instead, the number of dark growth zones of the individuals was related to the distribution of test diameters (Figure 6).

The numbers of these growth zones are plotted as Arabic numerals over each mm size increment of Figure 6. Some scatter and overlap of the size:age relationship occurred in the specimens larger than 50 mm. For example, an individual from the March 1970 collection had 14 dark growth zones, but was smaller than another specimen which had 12 growth zones. But the size-frequency peaks less than 40 mm in diameter generally were composed of specimens with the same number of growth zones. Two exceptions to this occurred. An 18 mm specimen from the October 1968 collection had only two dark growth zones while the remainder of those examined from the same size peak (mean at 27 mm) had three growth zones. The difference between the test diameter of the 18 mm individual and the mean of the peak was approximately 9 mm. This was similar to the mean size difference of the two large peaks of the September 1969 collection. The 18 mm urchin of the October 1968

collection may have represented a very small age class situated between the two larger age classes with means at 9 and 27 mm. Harding's graphical method for polymodal analysis of size-frequency distributions (1949) was used in an attempt to resolve a modal class near 18-19 mm, but none was demonstrated.

A 35 mm specimen from the September 1969 collection had four dark growth zones while other specimens of that peak had only three. Again this exception was near the extreme of the size range of the peak, 8 mm larger than the mean. This individual may have represented a small modal size class near 35 mm, but none was defined using Harding's method of graphical analysis. Thus, the means of the smaller size classes for the October 1968, September 1969, and November 1969 collections were found to be centered 9-10 mm apart. This value represented the increase in test diameter between spawning periods. The 9-10 mm test diameter increase did not agree with the 16-20 mm increase per year calculated from the shift of the mean size class test diameter with time. However, this increase did agree very closely with the growth rate calculated from the test plate growth zone counts.

The lack of agreement between the results of the two methods suggested that either the growth rate calculated for the two-month interval from September to November 1969 was substantially greater than the average annual growth rate, or that the size classes

determined from size-frequency analysis corresponded to semi-annual rather than annual recruitment classes.

Very little information about monthly or seasonal growth rate fluctuations was found for echinoids. Fuji (1967) obtained monthly measurements of the diameters of five year classes of the intertidal Strongylocentrotus intermedius (A. Agassiz) and found that each year class exhibited a steady annual growth rate which differed from the other year classes. Similar measurements of deep-water echinoids have not been made.

Reproductive Periodicity

The time and frequency with which new age classes of A. fragilis enter the population were determined by studying the gonadal condition of the adult urchins. Gonad index, determined from the gonadal biomass, has been widely used as an indicator of reproductive state and spawning period of echinoids (Boolootian et al., 1959; Giese, 1961; Boolootian, 1966; Fuji, 1967). For echinoids, the changes of gonad indices have agreed well with histological changes of the gonads and gametes (Boolootian, 1966; Fuji, 1967).

Gonad index has generally been calculated as the ratio of the wet weight of the gonad to the total wet weight of the animal (Boolootian, 1966). The accuracy of this procedure depended upon obtaining a meaningful wet weight value of the urchin. With A.

fragilis, accurate wet weights quite often could not be obtained because of crushing and abrasion of the tests and spines during trawling. Therefore test diameter rather than total wet weight of A. fragilis was used as an indication of size. This method also had the advantage of eliminating variation resulting from different blotting or draining periods prior to weighing the specimen.

Gonad indices were calculated for all A. fragilis specimens dissected. Usually gonad indices of 10-20 A. fragilis per trawl at any station were determined. Reproductive periodicity was studied only in specimens from the 200 m station, as the remaining stations lacked successive samples required for such a study.

An estimate of the minimum size at sexual maturity was established by plotting gonad wet weight as a function of test diameter for all 200 m station specimens examined (Figure 7). The maximum gonadal weight was very low for all animals less than 45 mm test diameter, then increased rapidly for specimens larger than 50 mm diameter. The actual minimum size at sexual maturity may be less than 50 mm, but an unexplained paucity of specimens with test diameters between 35 and 50 mm restricted the study of gonadal development in this size range. Only specimens larger than the estimated minimum size at sexual maturity were used for the study of reproductive periodicity.

Approximately monthly samples of A. fragilis were collected

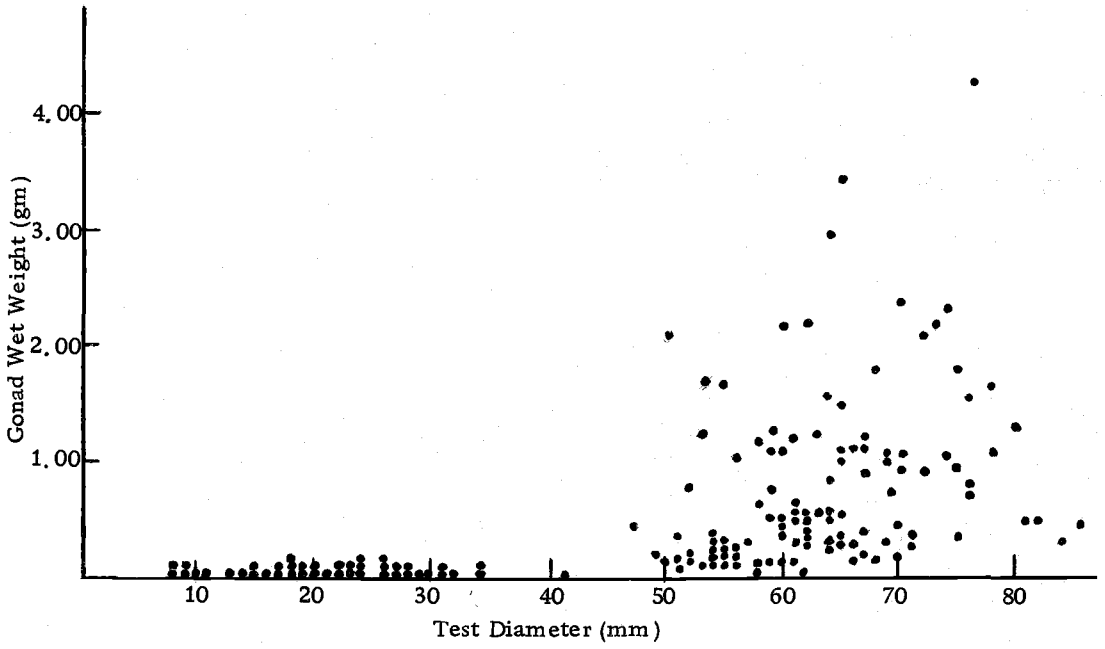


Figure 7. Variation of gonad wet weight with test diameter of A. fragilis from 200 m.

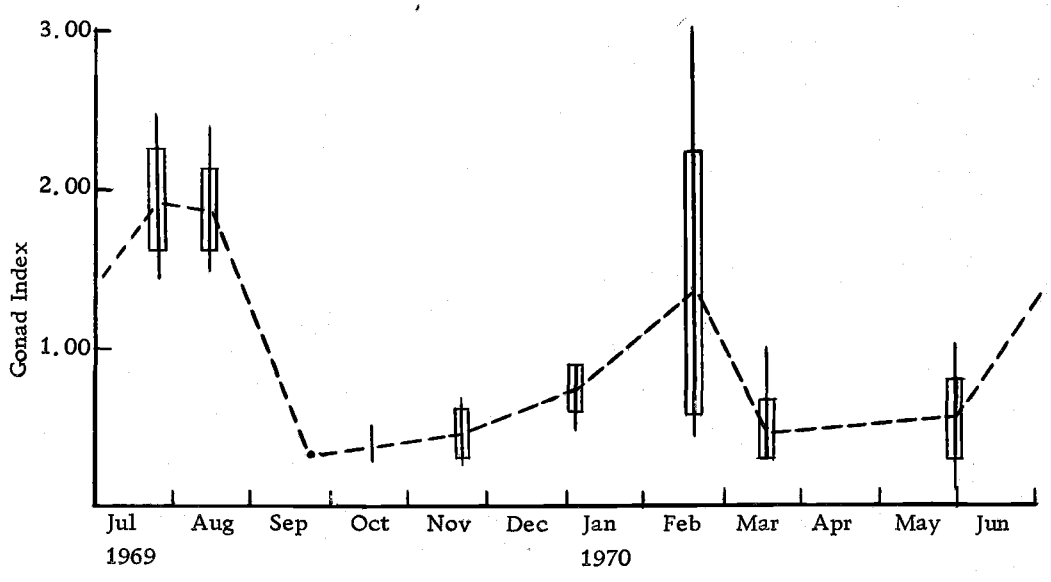


Figure 8. Monthly gonad index variation of A. fragilis greater than 49 mm in test diameter. Standard deviations and ranges of values are shown.

from the 200 m station between July 1969 and May 1970. Means of gonad indices with standard deviations and ranges of these samples are shown in Figure 8. The gonad indices show a marked semi-annual fluctuation in gonadal weight. This strongly indicates that two spawning seasons per year occur for this species; one in late summer-early fall (August-September), and one in late winter-early spring (February-March). Two age classes are thus spawned each year. Earlier studies on the reproductive periodicity of A. fragilis at Monterey Bay, California, established a single yearly spawning period which occurred between January and March (Boolootian et al., 1959; Giese, 1961; Boolootian, 1966). No references were made to an autumn spawning period.

Although the evidence is incomplete, Anderson (1964) stated that the phytoplankton off the coast of Oregon undergoes two blooms annually. A strong spring bloom follows the onset of upwelling. Surface primary production then decreases during the summer when nutrients in the surface waters are depleted. As the waters cool in the fall, stratification breaks down causing vertical mixing of nutrients and another phytoplankton bloom condition. The fall bloom is usually less pronounced than the spring bloom. As the nutrients in the surface waters are again depleted throughout the winter, primary production is reduced until the spring upwelling occurs (Anderson, 1964).

Boolootian et al. (1959) related spawning of A. fragilis in Monterey Bay to the annual onset of local upwelling. The spring and fall spawning periods off Oregon are also closely associated with periods of high primary productivity of the surface waters. The occurrence of spawning during surface phytoplankton bloom conditions may be advantageous in providing an abundance of food for the planktonic larvae of A. fragilis (Boolootian et al, 1959).

Gonad indices for A. fragilis from the stations below 200 m were calculated. Urchins from the 400 m station appeared reproductively mature. One specimen from this station had a high gonad index of 3.3. All specimens examined from 600, 800, and 1260 m had gonad indices of less than 0.3 and appeared reproductively immature. Although data for the deep stations are limited, gonad indices suggest that A. fragilis does not achieve sexual maturity below 400 m.

Published information on the rate and duration of the larval development of A. fragilis is lacking. Moore (1959) found that at equivalent temperatures the rates of development of A. fragilis and S. franciscanus (A. Agassiz) larvae were the same up to at least the five-day stage. After five days, his experiments were terminated. Details of the later larval development of A. fragilis are not known, but S. franciscanus larvae required 62 days to complete development from fertilization to metamorphosis (Johnson, 1930). The newly

metamorphosed S. franciscanus was less than 1 mm in diameter. If the rates of late larval development of both species remain nearly equal, larval metamorphosis and settling of A. fragilis would be expected approximately two months after spawning. For A. fragilis off Oregon, settling would probably occur in April-May and October-November of each year. At metamorphosis, the small A. fragilis are probably smaller than S. franciscanus, or less than 1 mm in test diameter.

Synopsis of Growth Rate Determinations

Agreement between the results of the different methods of growth rate determination can only occur if each age class is considered to be semi-annual and if new dark growth zones were added semi-annually. Moore (1935) considered the growth zones of Echinus esculentus to be bands of food-derived pigment which were seasonally incorporated into the growing skeletal material. The growth zones in the test plates of A. fragilis seem to reflect the semi-annual fluctuations of the amount of pigmented organic material available from surface productivity, suggesting that the two pigmented zones formed annually in the test plates are the result of semi-annual plankton blooms which occur in the surface waters. The data in Figure 2 suggest growth rates of 20 mm per year for 20 mm urchins and 18 mm per year for 30 mm urchins, with two

growth zones assumed to occur each year. These growth rates were essentially equal to those calculated from the shift of the mean size of each age class between September and November 1969 (Figure 6). Extension of the line intersecting the means of the November 30 mm peak and the September 27 mm peak to the October 1968 collection quite closely approaches the 9 mm mean of the small October 1968 age class. Further extension of the line to the y-intercept indicates that the age class which was 9 mm mean test diameter in October 1968 and 27 mm in September 1969 was spawned in early spring of 1968 and settled in late spring. The age class with a mean test diameter of 17 mm in September 1969 was probably spawned in autumn of 1968. This supports the late spring and late autumn times of metamorphosis and settling estimated from spawning periods and the assumed duration of the larval stage.

As shown in Figure 6, individuals of the age class spawned in spring 1968 had one dark growth zone in October 1968, two in January 1969, three in July and September 1969 and four in November 1969 and March 1970. One dark growth zone was apparently added between January and July and another between September and November of the same year. Both times are periods of high surface productivity. The non-pigmented zones separating the pigmented zones apparently reflect the decrease of surface primary productivity during the summer and winter months.

A refined theoretical growth curve for A. fragilis at 200 m was constructed using the size:age data shown in Figure 2. Two growth zones were considered to represent one year. The means and standard deviations of each "growth zone class" are shown in Figure 9.

The von Bertalanffy type of growth curve (Bertalanffy, 1938) was fitted to the mean growth zone lengths using the method of least squares described by Tomlinson and Abramson (1961). This is shown in Figure 9. The von Bertalanffy growth equation is:

$$\ell_t = L_\infty [1 - e^{-k(t - t_o)}]$$

where

ℓ_t = size at any age t,

L_∞ = the asymptotic size,

k = the rate at which the curve approaches the asymptote

t = age, and

t_o = age at size 0. For A. fragilis this value, which represents the time duration of larval stage, was assumed to be about 50-60 days or 0.15 years.

This equation has widely been used to describe the growth of organisms which asymptotically approaches a maximum size. This type of growth equation was successfully used by Fuji (1967) to describe

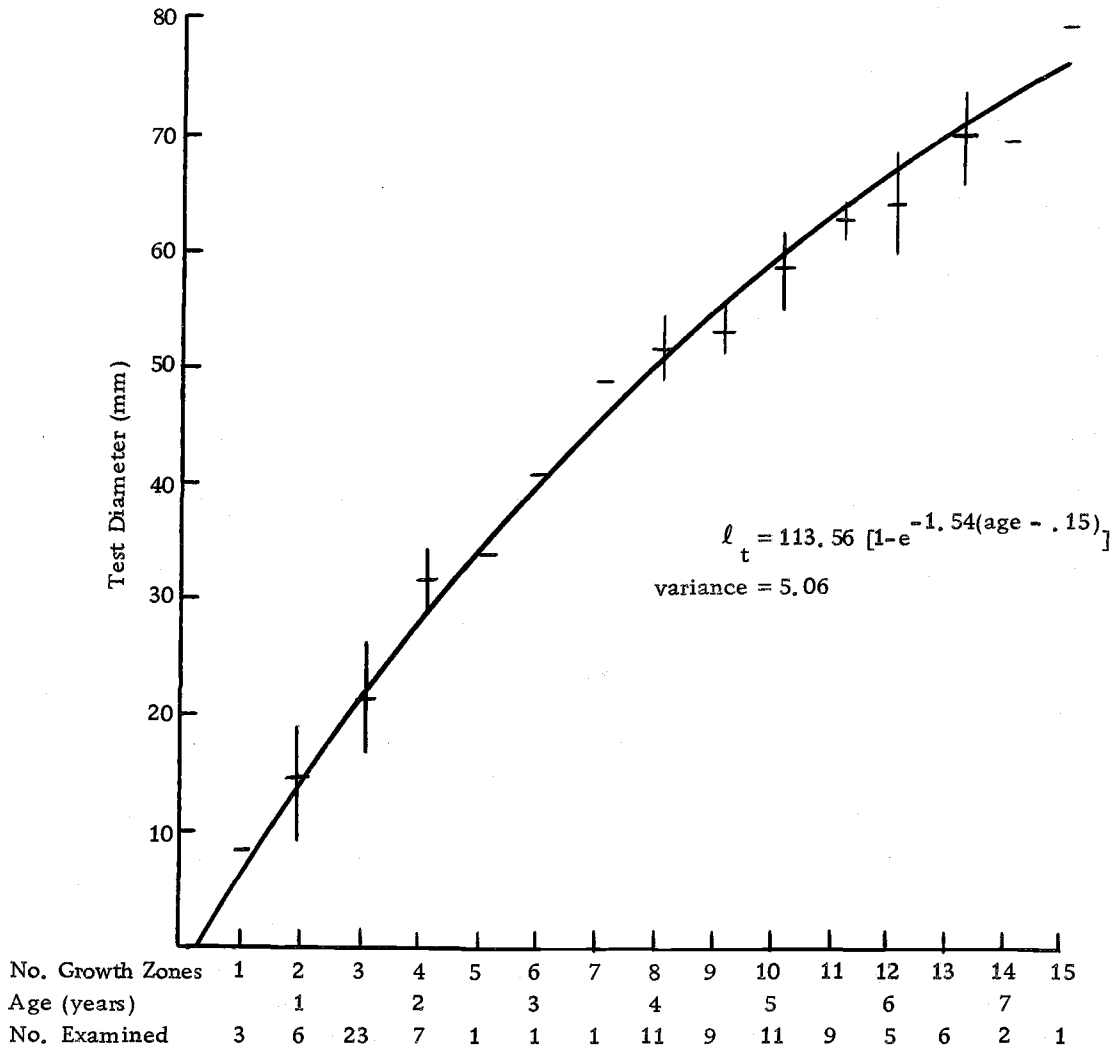


Figure 9. Least-squares fit of von Bertalanffy growth curve for A. fragilis from 200 m. Means and standard deviations of each growth zone class are shown.

the growth of S. intermedius.

Age data for A. fragilis from the remaining stations were insufficient to apply the Tomlinson and Abramson (1961) least squares method of fitting. Growth curves for these stations (using the data from Figures 3 and 4) were fitted by eye. These curves and the fitted curve of the 200 m station are shown in Figure 10. The age range of A. fragilis from the 100 m station was small and no growth curve was plotted; however it is apparent from Figure 2 that the growth rate of A. fragilis at 100 m is similar to that at 200 m. The growth curves for all the stations were assumed to have the same x-intercept, representing the duration of the larval stage.

Little apparent difference exists between the growth rates for A. fragilis from 100, 200, 400, and 600 m. But the growth rates for the 800 m and 1260 m stations are markedly less than the rates for the more shallow stations.

Although information on maximum ages for other strongylocentrotoid urchins is sparse and to a large extent based on circumstantial evidence, some comparisons may be made. Fuji (1967) was able to follow the growth of S. intermedius for five years. At that age, the urchins were near the maximum size for that locality. Swan (1961) found, using size-frequency studies and laboratory growth studies, that S. droebachiensis attained a test diameter of 52-54 mm in four years. Yet Jensen (1969) reported 11 annual

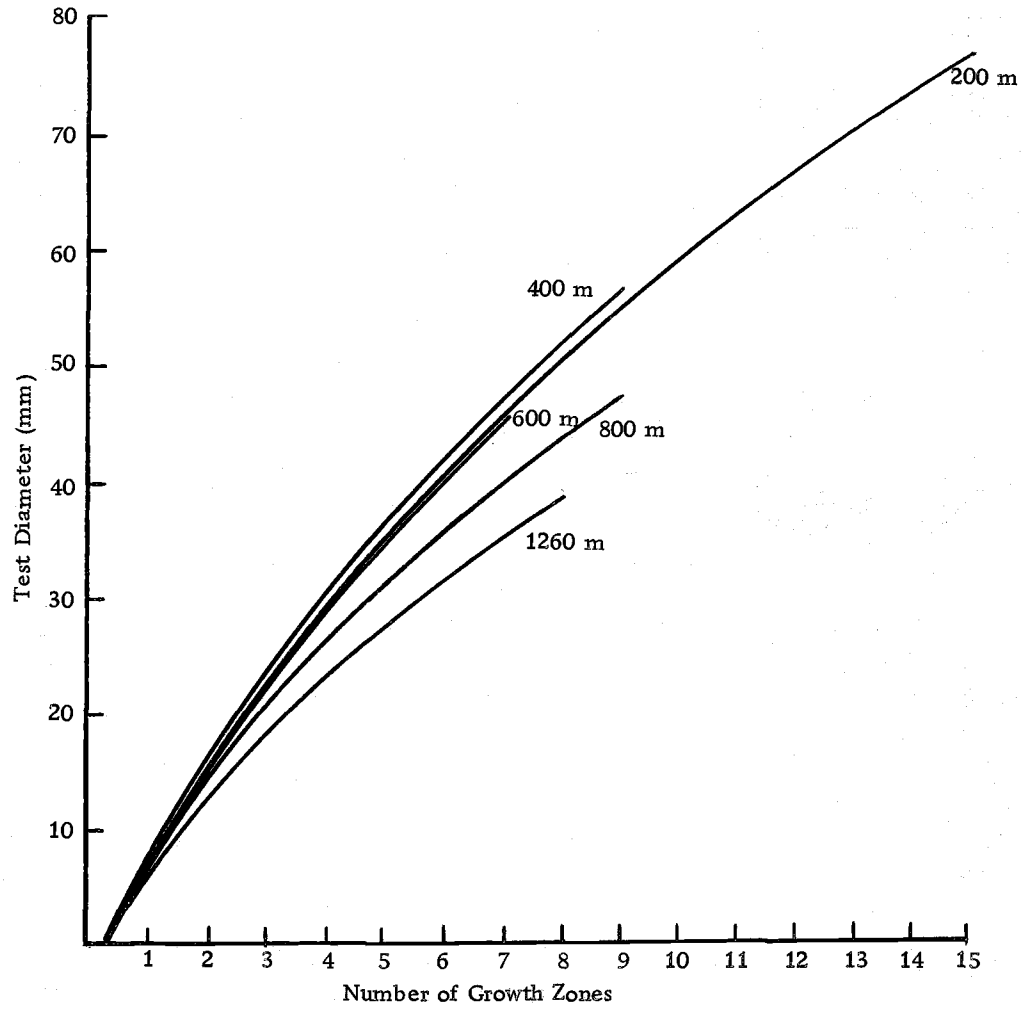


Figure 10. Compilation of A. fragilis growth curves from all stations. The 200 m growth curve is from Figure 9. Other curves fitted by eye to values shown in Figures 3 and 4.

growth zones in the test plates of a 51 mm S. droebachiensis specimen from Norway. Strongylocentrotus purpuratus are thought to live at least ten years (Ebert, 1967a).

The maximum A. fragilis age of seven and one-half years, determined from growth zone counts of an 80 mm specimen, compares favorably with the reported maximum ages of other strongylocentrotoids. Allocentrotus fragilis larger than 80 mm, and presumably older than seven and one-half years, are not uncommon. Individuals up to 88 mm in test diameter were collected (see Figure 6). Thus this species may be expected to live for at least ten years.

Skeletal Magnesium Content

The skeletal material of echinoderms is composed primarily of $CaCO_3$ with lesser amounts (usually less than 15%) of $MgCO_3$. In most echinoderms a general trend exists for individuals from warm waters to have greater amounts of Mg incorporated into the calcite crystal lattice than for individuals from colder waters (Clarke and Wheeler, 1922; Chave, 1954; and Pilkey and Hower, 1960). This trend is thought to reflect higher rates of growth and metabolism of warmer water echinoderms (Weber, 1969b).

The reduced growth rates of A. fragilis at the deeper end of its bathymetric range suggest that the skeletal Mg concentration might be less than that of specimens from 200 m. However, preliminary

skeletal composition analyses of A. fragilis by Sumich (1969) indicated that specimens from 800 m had a higher mean skeletal Mg content than did specimens from 200 m. These findings did not indicate whether the differences in skeletal Mg content existed throughout the size range of A. fragilis at each depth, or merely reflected a variation of skeletal Mg content with size or age; with the smaller and younger urchins at 800 m having more skeletal Mg than the larger, older urchins at 200 m.

To resolve this problem, the $MgCO_3$ content of the test plates of A. fragilis from 200, 800, and 1260 m was determined. The mean skeletal $MgCO_3$ content was again found to increase with depth (see Table 2). These values are very close to the 6.95% $MgCO_3$ reported by Vinogradov (1953) for this species.

Table 2. Results of Mg analysis of A. fragilis test plates.

Depth in m	Number of Specimens	Mean Test Diameter in mm	Mean Percent $MgCO_3 \pm SD$
200	56	47.1	6.9 ± 0.5
800	19	32.3	7.0 ± 0.3
1260	5	31.0	7.3 ± 0.3

The regression lines of the skeletal Mg values plotted against age, as determined from growth zone counts, were plotted for each depth (Figure 11).

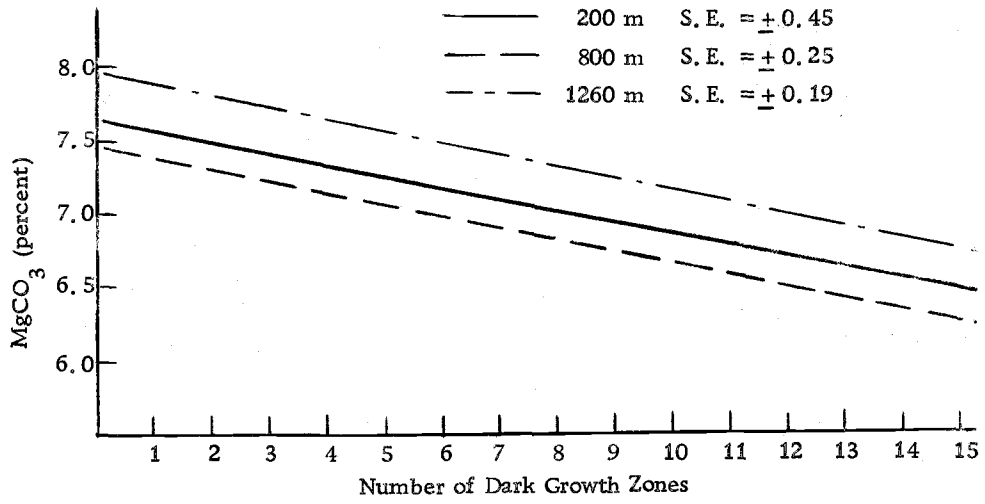


Figure 11. Variation of A. fragilis skeletal Mg content with age at 200, 800, and 1260 m.

These results indicated that either the amount of Mg incorporated into newly-formed calcite decreased as the urchin grew older or that Ca ions were gradually being substituted for Mg ions which were already incorporated into the calcite lattice. If the former were true, the older skeletal test plates near the peristome should contain more Mg than the younger plates nearer the apical system; but if Ca was gradually substituted for Mg in the calcite lattice, the older plates should contain equal or less Mg than the younger plates.

That older A. fragilis incorporate less Mg into the skeletal calcite was shown by a series of Mg determinations along the length of complete interambulacral test plate columns (such as shown in

Figure 1). Test plates of a 66 mm specimen from 200 m and of a 27 mm specimen from 1260 m were used. The interambulacral plates were numbered from the peristomeal margin. The plates were treated in groups of three to facilitate analysis. The results of the Mg analyses of these plates are shown in Table 3.

Table 3. Percent $MgCO_3$ of A. fragilis test plate groups along an interambulacral column. Plates are numbered from the peristomeal margin.

Plate No.:	1-3	4-6	7-9	10-12	13-15	16-18	19-21
200 m	7.1	6.9	6.5	6.4	6.4	6.2	5.9
1260 m	8.3	7.1	6.4	5.9	6.0		

The data in Table 3 show a definite decrease in Mg content from the oldest to the newest plates. This, in conjunction with the data shown in Figure 11 indicate that, as A. fragilis ages and the growth rate decreases, less Mg is incorporated into the skeletal material being formed. This agrees with statements by Dodd (1967) and Weber (1969b) that echinoids with slower growth rates generally have smaller amounts of skeletal Mg. Mechanisms for this are not known, but Weber (1969b) proposed that Mg is incorporated into the calcite lattice by random substitution for Ca at the site of calcification. The Mg ion, however, is less stable than the Ca ion in the crystal lattice. Thus, in slowly forming calcite (viz., slowly growing urchins), more Mg may be excluded from the calcification site prior to actual

calcite formation by the more stable Ca ion. Conversely, in rapidly forming calcite more Mg may be incorporated into the calcite lattice prior to Ca exclusion.

The vertical separation of the regression lines (Figure 11) for the three depths is less than the standard error of the 200 m regression line. Thus little difference in skeletal Mg content existed between specimens from different depths which were the same age. Most of the skeletal Mg variation of A. fragilis was the result of less Mg in older specimens. As the slopes of the three regression lines in Figure 11 are essentially equal, the difference in growth rate between A. fragilis from the 200, 800, and 1260 m stations apparently did not affect the Mg composition of the skeletal calcite.

Discussion of Environmental Factors

The difference in relative abundances, growth rates, maximum sizes, maximum ages, and reproductive condition between stations suggest that environmental conditions are most optimal for A. fragilis at the 200 m station. Of the many environmental factors which might possibly affect the growth rate and longevity of marine invertebrates, Hallam (1965) listed the following as being important: 1) turbidity, 2) agitation, 3) population density, 4) salinity, 5) water temperature, 6) oxygen tension, and 7) food supply.

In considering the growth rates of A. fragilis, some of these

factors can be dismissed as unimportant. At depths greater than 100 m, turbidity is not obvious from bottom photographs and therefore probably has no effect on the growth rate of A. fragilis. Agitation must also be nearly non-existent, as shown by the lack of turbidity. Population density of A. fragilis was greatest at 200 m where the growth rate is highest. Apparently, A. fragilis does not achieve a density great enough to cause a reduction of its growth rate, at least relative to A. fragilis to other depths.

The salinity, temperature, and oxygen tension of the bottom water at each of the six stations are shown in Figure 12. There is a general trend for all three factors to change with increasing depth or distance offshore. The salinity values increase only 1.7‰ from 200 to 1260 m (Figure 12). This difference probably can be considered physiologically unimportant. The temperature and dissolved oxygen values decreased sharply with increasing depth; with the dissolved oxygen values reaching a minimum at the 800 m station, then increasing slightly to the 1260 m station.

Bottom Water Temperature

The mean bottom water temperature at 200 m was 7.5°C decreasing to 3.5°C at 1260 m (Figure 12). Although a corresponding decrease in the growth rate might be speculated as the metabolic rate

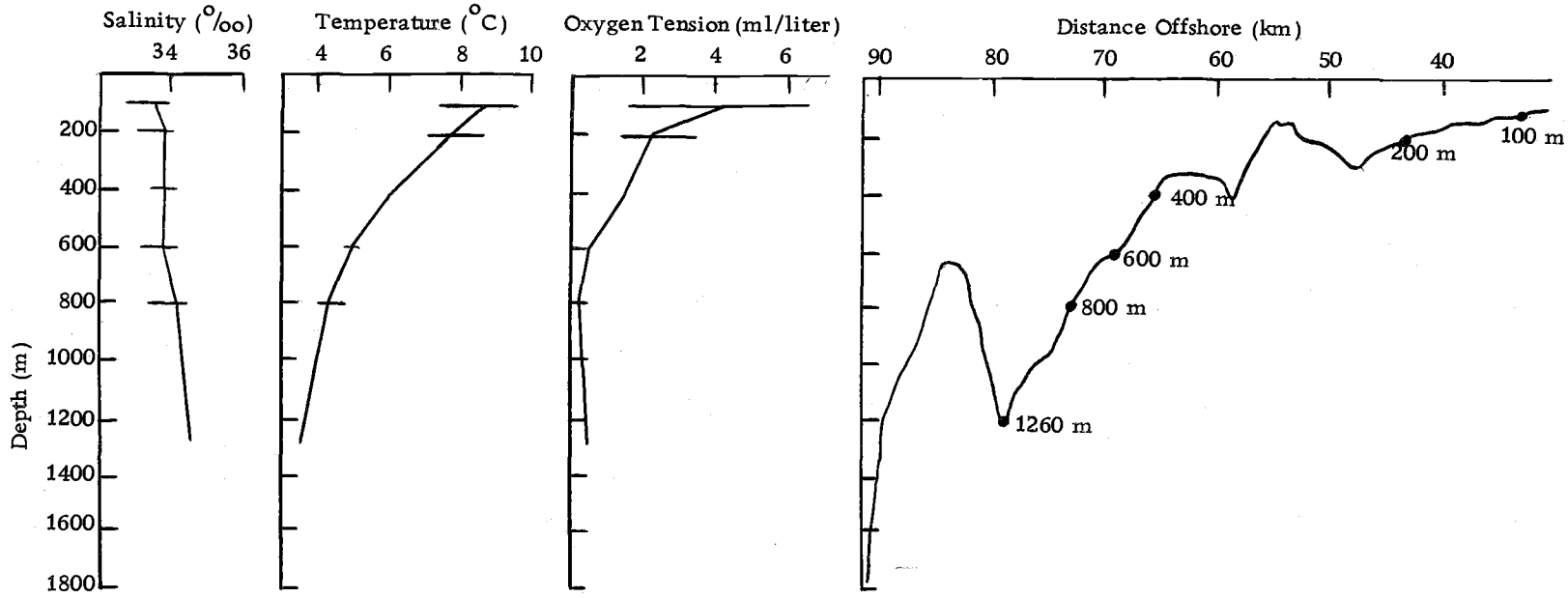


Figure 12. Variation with depth of bottom water salinity, temperature, and oxygen tension values. Horizontal lines represent ranges of the values. Station locations are shown at right.

of A. fragilis, determined by the rate of oxygen consumption, was found to be largely temperature dependent (Ulbricht, 1970); the temperature difference between 200 and 1260 m is relatively small and temperature acclimation of A. fragilis has not been studied.

Bottom Water Oxygen Tension

Echinoids have been described as oxygen consumption conformers (Farmanfarmaian, 1966), the rate of oxygen consumption being dependent on the ambient oxygen tension. Recently, Johansen and Vadas (1967) found that the oxygen consumption rate of several species of Strongylocentrotus was independent of ambient oxygen tension values down to 60-70 mm Hg, but was dependent on ambient oxygen tension below this. Thus, at least for low environmental oxygen tension conditions, many echinoids, and presumably A. fragilis, are oxygen consumption conformers. Metabolic rate decrease and a corresponding growth rate decrease of A. fragilis at the deep stations may result from the very low bottom water oxygen tension values (less than 0.5 ml/liter) at the deep stations.

Gut Content Analysis and Food Availability

The stomach contents of A. fragilis from all stations consisted of food "balls" or pellets 0.7 to 3.0 mm in diameter. Microscopic examination of these pellets revealed shreds of macroalgae,

diatom tests, foraminifers, chitinous remains of zooplankton, and a few skeletal parts of small bottom invertebrates. A similar description of the gut contents of A. fragilis inhabiting a rocky bottom in Monterey Bay was given by Boolootian et al. (1959). Some sand and other sediment grains were often incorporated into the food pellets. The gut content composition did not differ visibly between stations. The organic dry weight of the gut contents of A. fragilis from the 200 m station collections varied from 20.1% to 32.5%. Values for the other stations were within this range.

For comparison, the gut contents of the heart urchin, Brisaster latifrons (A. Agassiz), were analyzed. Brisaster latifrons, collected at five of the six stations at which A. fragilis were taken, is infaunal and thought to ingest sediment for nutrition (McCauley and Carey, 1967). Microscopic analysis of B. latifrons gut contents revealed material indistinguishable from surrounding sediment. The organic dry weight of B. latifrons gut contents was 7.5% of the total dry weight at 200 m and 14.2% at 800 m. The greater organic fraction and obvious visual difference of A. fragilis gut content presumably reflect a particulate detrital feeding habit, as opposed to a sediment ingesting habit of B. latifrons.

This was further borne out by unpublished findings of A. G. Carey and C. L. Osterberg (cited in McCauley and Carey, 1967) that radioactive ^{65}Zn was concentrated to a greater extent by A.

fragilis than by B. latifrons. Differences in ^{65}Zn uptake were thought to reflect the different feeding habits of the two species, as ^{65}Zn accumulates in a thin layer at the sediment-water interface (Jennings, Cutshall, and Osterberg, 1965).

The amounts of detrital material available seasonally or annually at depths of 100-1260 m off Oregon have not been determined. But there is a general trend for areas in deeper water and further offshore to receive less effects of surface productivity (Sverdrup, Johnson, and Fleming, 1942, p. 808-809; Hallam, 1965). Thus, if other factors such as competititon are similar, less detrital material would be available at the deeper stations and growth of A. fragilis could be limited.

Variation of water temperature, oxygen tension, and food supply between the shallow and deep stations may individually or together explain the difference of A. fragilis growth rates at these stations. However, these hypotheses must be considered tentative and in need of substantiating data.

SUMMARY OF CONCLUSIONS

The use of test plate growth zones as an indicator of age is an effective and simple method of obtaining age estimates for A. fragilis. This method is especially useful for older urchins when size-frequency distributions are confusing.

Two dark growth zones are formed in the test plates of A. fragilis each year. The formation of these zones correspond to the periods of highest primary productivity in the surface waters. Allocentrotus fragilis spawns in the spring and the fall, giving rise to two age classes annually. The occurrence of spawning also seems to be closely associated with periods of high surface productivity.

Off Oregon, A. fragilis is more abundant, and achieves greater size and age at 200 m than deeper within this species' bathymetric range. Growth rate is least at 1260 m.

The Mg content of A. fragilis skeletal calcite was found to decrease with increased age of the specimen. Also, the younger test plates contained less Mg than older plates. The decrease of skeletal Mg was related to the decrease in growth rate of the older urchins. Little difference in skeletal Mg content of A. fragilis at different depths was found.

The low water temperatures and dissolved oxygen concentrations taken together with a decreased food supply at the deep stations

are suggested as being responsible for decreased growth rates and reduced longevity of A. fragilis in the deeper parts of its bathymetric range.

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