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Growth and Size of the Tropical Sea Cucumber *Holothuria* (*Halodeima*) *atra* Jäger at Enewetak Atoll, Marshall Islands[1]

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ABSTRACT: In September 1975, 5031 sea cucumbers (*Holothuria atra* Jäger) were tagged with tetracycline and returned to a gutter on the seaward reef bench of Ananij Island, Enewetak Atoll, Marshall Islands. A sample of 184 individuals was collected in September 1976. Animals were dissected and plates of the calcareous ring were examined with ultraviolet radiation for tetracycline lines. Based on 18 tagged individuals, the Brody–Bertalanffy growth constants are: $K = 0.11$ and $P_\infty = 0.89$ cm (for interradial plates). Length (L, cm) and weight (W, g) of individuals are related to plate size: $L = 36.35P$ and $W = 1950P^3$, giving maximum size as 32 cm and 1352 g. Length frequency distributions for the population did not change from 1975 to 1976. A preliminary estimate of annual loss is 50 to 70 percent of the total population based on the growth parameters and population size structure. *Holothuria atra* shows asexual reproduction by transverse fission, which appears to be the major source of recruitment at Ananij rather than from the plankton.

ON MANY TROPICAL REEFS, sea cucumbers are abundant and may attain densities in excess of 200/m^2 (Bakus 1968). But they are very difficult to study as populations and very few estimates of growth and death rates of holothurians are in the literature. Edwards (1908) presents growth data for newly metamorphosed *Holothuria floridana* Pourtalés, but only up to a size of 4 mm and an age of 75 days under laboratory conditions. Rutherford (1973) presents growth data of newly recruited *Cucumaria pseudocurata* to an age of 1 year and gives estimates of loss rate for animals less than 1 year old. Fish (1967) and Buchanan (1967) discuss growth and mortality of *Cucumaria elongata* and calculate rates based on data for animals of many ages.

The purpose of this paper is to call attention to a technique that shows some promise as a means of studying growth of holothurians and so, somewhat indirectly, of estimating mortality and population turnover. The experimental animal was *Holothuria* (*Halodeima*) *atra* Jäger, an abundant and widely distributed Indo-Pacific aspidochirote, which was studied at Ananij Island, Enewetak Atoll (11°28′ N, 162°24′ E). A second purpose of this paper is to present information about growth, death, and size for this species.

Holothuria atra is very abundant at certain areas of Enewetak Atoll. Bakus (1973) shows a picture with a density of 5 to 35 individuals /m^2 at what he calls Anayaanii Island. This is the same island Johannes *et al.* (1972) call Japtan and which is now called Ananij. Military charts list this island as Bruce. The area where cucumbers were studied seems to be the same area illustrated by Bakus (1973); it is a reef gutter on the seaward side of Ananij. The vegetated portion of Ananij is narrow at the southern end and has an enclosed inlet on the seaward side bordered by the main part of the island on the north and by a narrow peninsula of vegetated land (covered nearly exclusively by *Pemphis aci-*

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dula) on the south. The gutter where sea cucumbers were tagged is to the south of this vegetated peninsula. At low tide the gutter is separated from the sea, but at high tide there is a strong current flowing from east to west and deflected to the south and around the southern tip of the island. The bottom of the gutter is an eroded reef with a layer of 0 to 5 cm of coral sand.

METHODS

Tetracycline is known to be taken up by the growing surfaces of echinoderm skeletons (Kobayshi and Taki 1969, Pearse and Pearse 1975, Taki 1971) and has been used to analyze growth in echinoids (Ebert 1977). Holothurians have, in addition to small ossicles in the body wall, a series of larger plates at the beginning of the pharynx called the calcareous ring (Hyman 1955). The plan was to tag these plates of the calcareous ring with tetracycline, measure growth of these plates, and use this information to describe growth of other body parts of *H. atra*.

On 20 and 21 September 1975, 5031 individuals were tagged with 0.2 ml of a solution of 1 g tetracycline hydrochloride dissolved in 100 ml seawater. The solution was injected into the body with no attempt to select a definite site. Length of the first 1015 sea cucumbers was measured to the nearest 0.5 cm. Holothurians are able to change length and weight, so the only hope of obtaining reasonable comparisons of size was to try to standardize the handling technique. Animals were collected and placed in a plastic laundry basket and when the basket was full (about 200 animals) it was moved about 15 meters to the place where the animals were measured as quickly as possible. Sea cucumbers were placed on a fish-measuring board and length was read within 10 sec. There appeared to be no difference in tendency to contract or relax between animals at the top or bottom of the basket.

On 22–28 September 1976, 610 animals were measured in the field; 116 were brought back to the marine laboratory on Enewetak Island where they were weighed and dissected to remove the calcareous ring. An additional 68 animals were measured and dissected in the field, so measurements would be on animals handled the same as those used to determine size structure of the population.

Rings that were dissected from *H. atra* were treated with 5 percent sodium hypochlorite to remove organic material, washed, dried, and then examined with ultraviolet radiation under a dissecting microscope.

RESULTS

Of the 184 animals collected in 1977, only 18 individuals showed clear tetracycline marks. I have reason to believe, however, that all animals I dissected had been tagged. Sea cucumbers were very highly concentrated in the areas where they had been released in 1975, but densities were very low in the surrounding areas where individuals had been collected. The recovery of only 10 percent with a clear mark, I believe, is due to only 10 percent of the population actually growing at the time of tagging or to regeneration rather than mixing of tagged and untagged sea cucumbers. Tetracycline hydrochloride is both temperature- and *p*H-sensitive; the higher the *p*H and temperature, the faster breakdown occurs (Barnes 1971). Breakdown on a tropical reef probably takes no longer than 1 day, so if an animal is not growing at the time it is tagged, it will not be marked. Also, animals that regenerated a complete ring after tagging would lack a mark.

Lengths of the interradial pieces of the calcareous ring were measured using an ocular micrometer [see Hyman (1955), Figure 56, for an illustration of plates of the calcareous ring]. Length of the plate is defined as the distance along the radius of the ring (i.e., perpendicular to the long axis of the individual). Two to four plates were measured in each individual. In a sample of 30 animals with average plate lengths of 0.426 cm and SD = 0.059, the average standard error of plate length within individuals was 0.024 cm, with an associated standard deviation of 0.015 cm. It is apparent that variation of

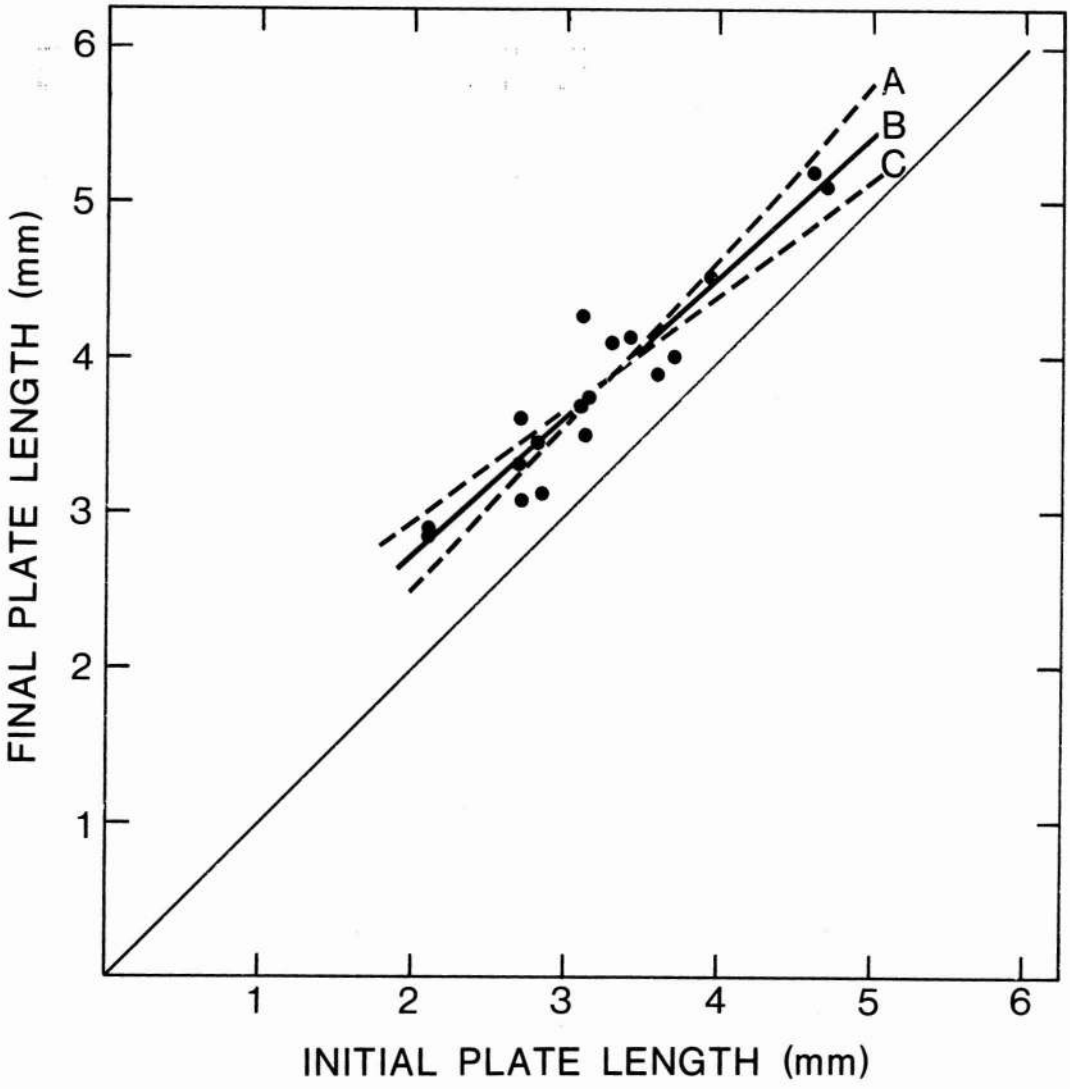

FIGURE 1. Ford–Walford plot of tetracycline-tagged interradial plates of the calcareous ring of *Holothuria atra*, Ananij Island, Enewetak. Plate length in 1976 (final plate length) is plotted against the length of the internal glowing image (initial plate length, i.e., the size in 1975). Dashed lines A and C are 95 percent confidence limits of slope. The line drawn at 45° is the line of zero growth.

plate size within an individual was substantial and also that some individuals were much less variable than others.

In measuring tetracycline marks, length of the glowing image represents the size of the plate at the time of marking in 1975. Figure 1 shows average values of size of interradial plates in 1975 and 1976. A regression of S_{t+1} versus S_t, the well-known Ford–Walford plot, was used to estimate growth parameters of the Brody–Bertalanffy growth equation [see, for example, Gulland (1969), Ricker (1975), or, for application to echinoderms, Ebert (1975)].

The parameters and 95 percent confidence limits of the Ford–Walford line are: slope = 0.893 ± 0.165, intercept = 0.095 ± 0.053 cm. A slope of 1.0 with an intercept of 0.06 cm is within the 95 percent confidence limits.

Parameters of the Brody–Bertalanffy equa-

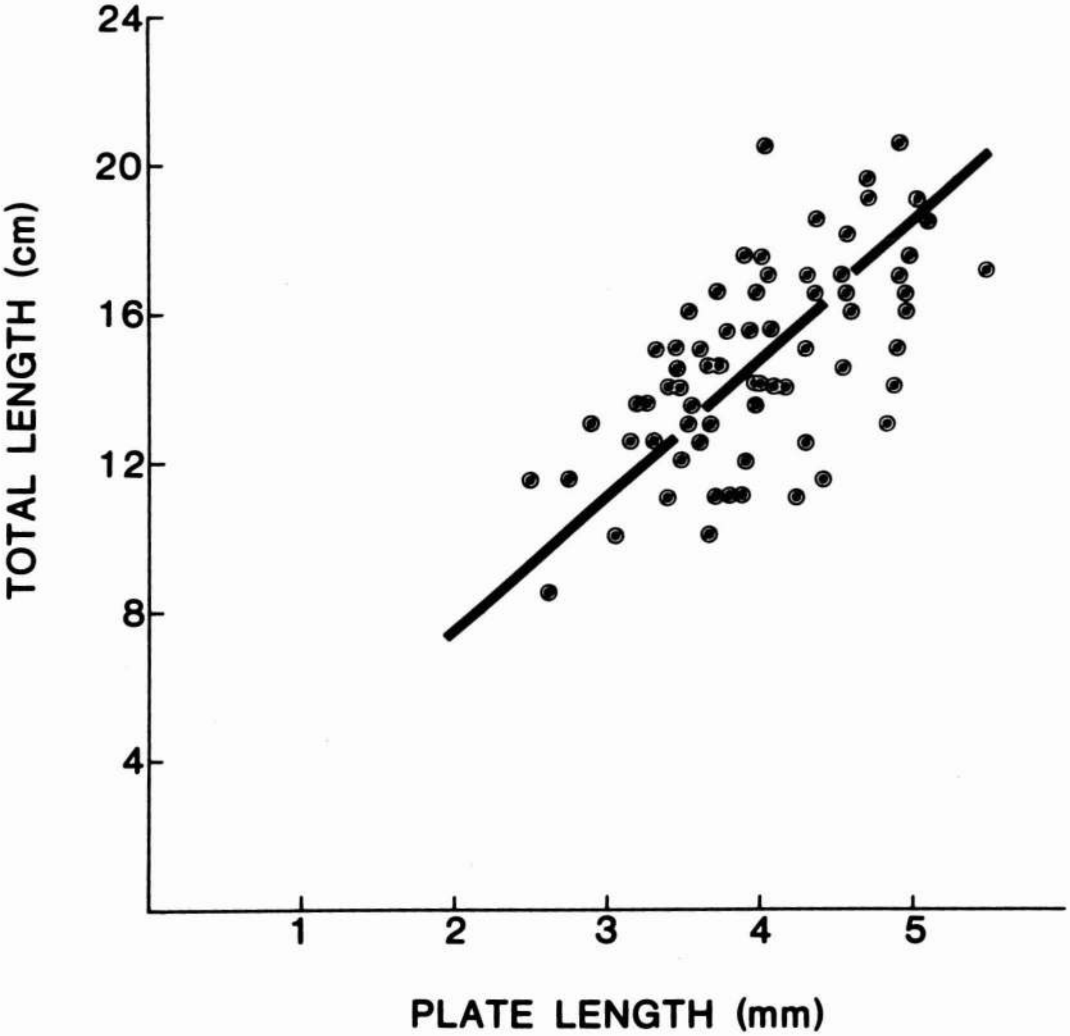

FIGURE 2. Total length of *Holothuria atra* individuals at Ananij Island versus length of interradial plates. Total length measurements were made in the field using the same handling procedure as was applied in constructing length frequency distributions.

tion are calculated from the slope and intercept of the Ford–Walford line. Because the regression line rotates around the point ($\overline{S}_t$, $\overline{S_{t+1}}$), the line with the largest slope also has the smallest intercept; in terms of Brody–Bertalanffy constants, a large growth rate constant, K, will be associated with a small asymptotic size, P_∞. Using the regression line (B) in Figure 1, parameter estimates of the growth equation are $K = 0.106$ and $P_\infty = 0.891$ cm. The growth equation for plate length, P, measured in centimeters, and time, t, in years, is

$$P_t = 0.891(1 - be^{-0.106t}) \qquad (1)$$

The parameter b is equal to $(P_\infty - P_0)/P_\infty$, where P_0 is the size of a plate in a newly settled individual. I have assumed that $P_0 = 0.0$, so $b = 1.0$. Actual size of a plate in such an individual is probably about 0.001 cm, so b would be equal to 0.999. Assuming $b = 1.0$ does not affect positioning of the curve to a significant degree. For a slope of 0.728 (the lower 95 percent limit) and intercept of 0.148 cm, the parameters would be $K = 0.317$ and $P_\infty = 0.542$.

The parameter K will have a negative sign and there is no asymptotic size when the Ford–Walford line has a slope greater than 1.0. The Brody–Bertalanffy equation may still be used, but should be rewritten without using the symbol P_∞:

$$P_t = \frac{C(1 - be^{-Kt})}{1 - e^{-K}} \qquad (2)$$

where C is the y intercept and e^{-K} is the slope of the Ford–Walford line. The upper 95 percent limit of the regression coefficient in Figure 1 is 1.058, so $K = -0.056$ and $C = 0.042$. Finally, with a slope of 1.0, the growth equation is

$$P_t = 0.06t \qquad (3)$$

Growth was expressed in terms of total body length by establishing the relationship between body length and plate length. Figure 2 plots length for the 68 individuals measured in the field against plate length, and shows a zero intercept regression line (Bliss 1967). Where L is total length in cm, P is plate length in cm, and with the 95 percent confidence interval of the slope, the relationship is

$$L = 36.35(\pm 1.30)P \qquad (4)$$

Plate length also was related to total wet weight using 99 animals brought back to the marine laboratory at Enewetak Island. A regression line was fitted with zero intercept, with weight, W, in grams, and with the 95 percent confidence interval for the slope:

$$W = 1950(\pm 178)P^3 \qquad (5)$$

Growth curves for total length are shown in Figure 3. An average animal 5 years old would be between 8.5 and 15.5 cm long and weigh between 25 and 156 g; a 9-year-old individual would be between 17.0 and 20.0 cm long and weigh between 200 and 320 g.

Length frequency distributions for 1975 and 1976 are shown in Figure 4. Distributions for both years are unimodal and are not skewed. The two distributions were compared for differences in location of the mean or in shape using the Kolomogrov–Smirnov test (Tate and Clelland 1957). The maximum difference between the two distributions was 4.9 percent at the interval 11 to 12 cm. The significant difference with $\alpha = 0.05$ is 6.97 percent ($n_1 = 1015$; $n_2 = 610$), so the null hypothesis H_0 is not rejected and I conclude that the size distribution had not changed from 1975 to 1976.

Size distributions can be used in conjunction with growth parameters to obtain initial estimates of mortality or loss. Van Sickle (1977) provides an easily applied technique for estimating m, the instantaneous loss rate:

$$m = -gs + K \qquad (6)$$

where s is the slope of the right descending limb of the semi-$\log_e$ transformed size distribution (ln N versus size), g is the average growth rate of animals from the mode to the largest indivduals, and K is the growth constant.

Using combined size distributions from Figure 4, the slope of ln N versus size is -0.549. For line B in Figure 3, the average growth rate is 1.66 cm/year for animals 13 to 22 cm long. The value of K is 0.106; accordingly, $m = 1.02$. The average growth rates for lines A and C are 2.45 and 0.60 cm/year, which give instantaneous loss rates of 1.29 and 0.65, respectively. Annual loss rates are 72, 64, and 48 percent for lines A, B, and C, respectively, in Figure 3.

Loss of individuals is only in part due to mortality. *Holothuria atra* appears to be capable of asexual reproduction by transverse fission. This has been reported in the literature (Bonham and Held 1963) and reviewed by Bakus (1973) for other holothurians. I collected several small *H. atra* at Ananij between 5.0 and 7.0 cm long, all of which were very wide for their length and so fit the description given for small individuals by Bonham and Held (1963). One individual had very large plates in its calcareous ring, one had exceptionally small

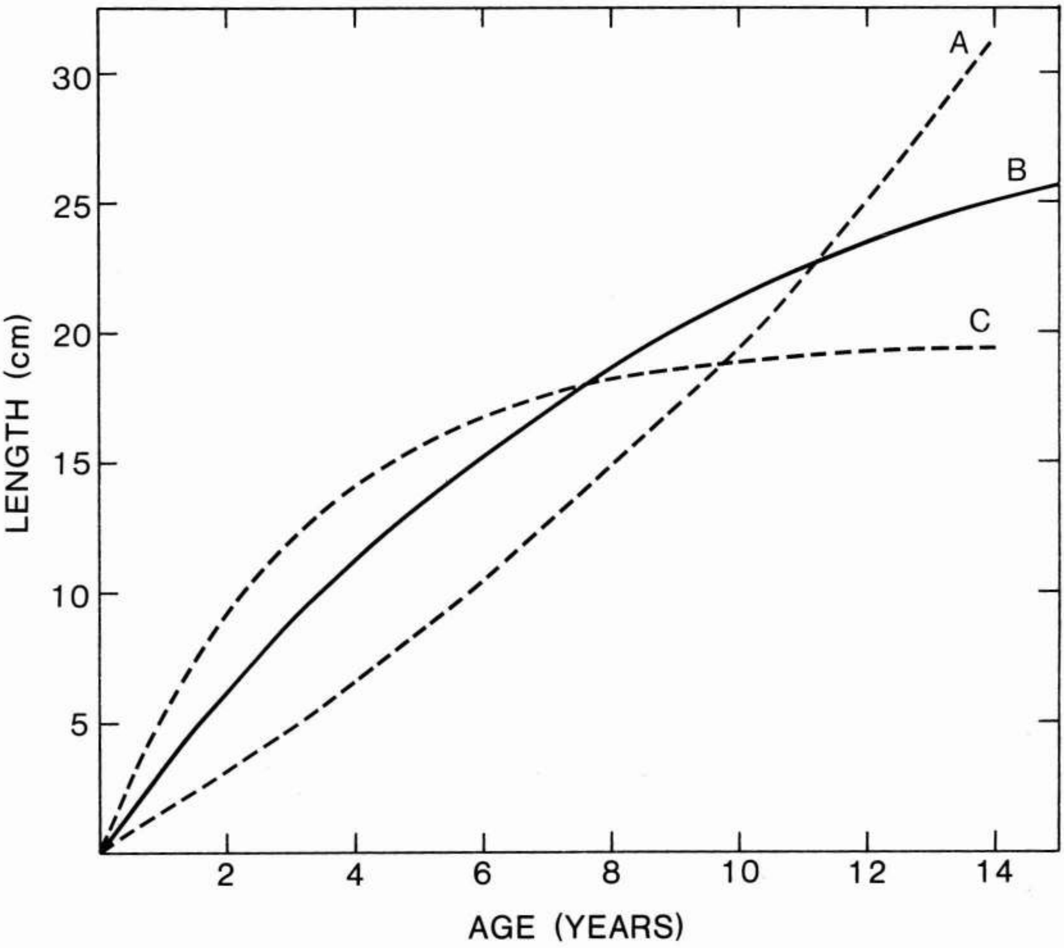

FIGURE 3. Brody–Bertalanffy growth curves for *Holothuria atra* using constants obtained from Figure 1 and the conversions of interradial plate to total length. Line A is from A in Figure 1, line B is from B in Figure 1, etc.

plates, and one had no ring at all. A reasonable interpretation is that the individual with the large plates was the front end of a divided individual; the other two were back ends in different stages of regeneration.

Asexual reproduction has important consequences when interpreting the loss rates. In part, loss of large individuals from a population would not be through death but by fission.

DISCUSSION

Use of tetracycline shows some promise for tagging the growing pieces of the calcareous ring in sea cucumbers. The two major problems with the technique are (1) the necessity of sacrificing the animals in order to determine whether they were tagged and also to measure the amount of growth, and (2) an animal showing zero growth during a year cannot be distinguished from an individual that was tagged and not growing but grew at some other period during the year, or from an individual that regenerated its plate or never was tagged.

As indicated by Bakus (1973), the existence of asexual reproduction by transverse fission complicates the analysis of size distributions. Sizes that are normally distributed are shown by Bonham and Held (1963) for *H. atra* at

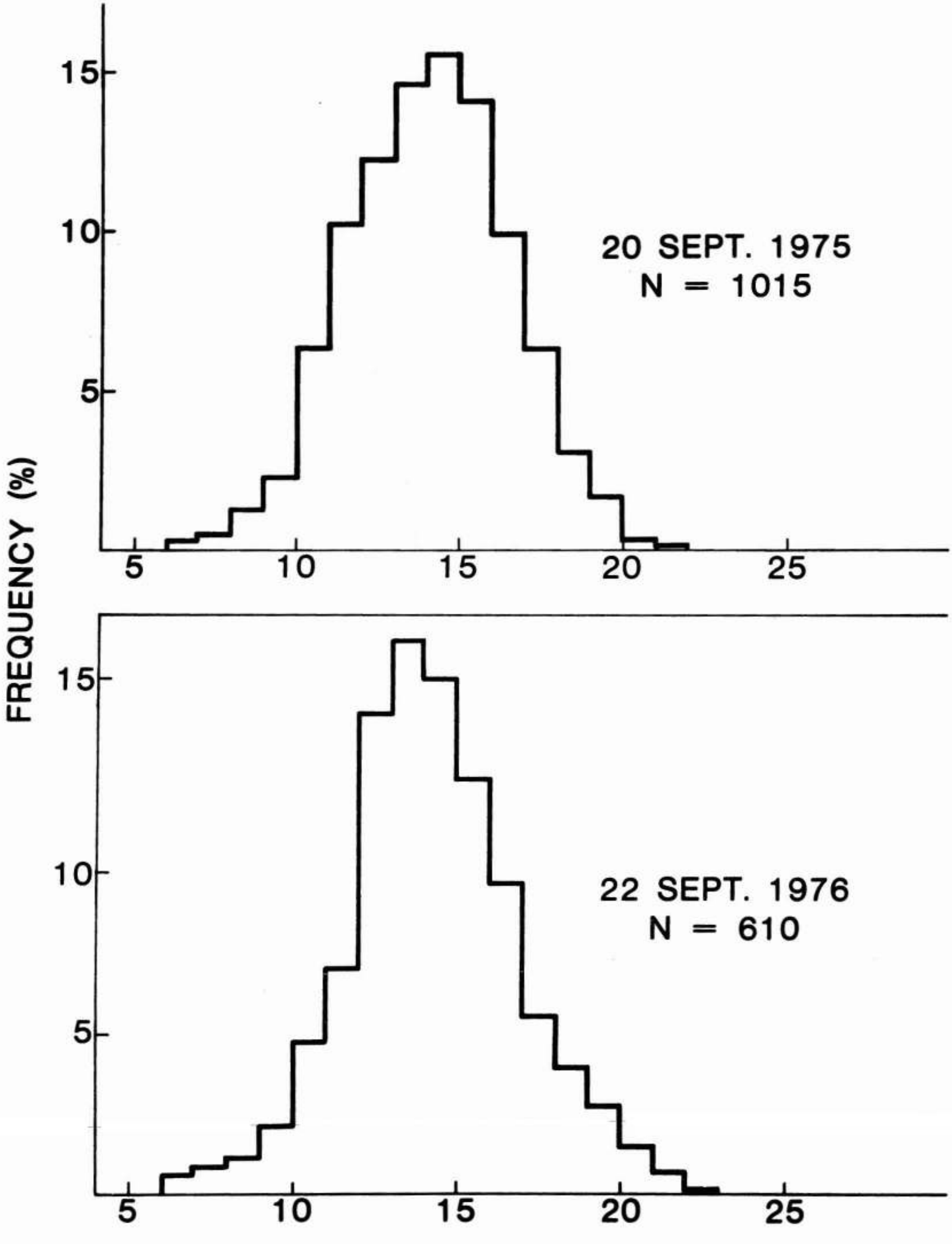

FIGURE 4. Length frequency distributions for *Holothuria atra* at Ananij Island for 1975 and 1976. These distributions are not significantly different ($\alpha = 0.05$) using the Kolomogrov–Smirnov two-sample test.

Kabelle Island, Rongelap Atoll. The mean length at Kabelle was about 13.0 cm, with minimum and maximum values of 5.0 cm and 25.0 cm, respectively. It is a distribution that looks much like the size structures of the cucumbers at Ananij. Also, like *H. atra* at Enewetak, they found very large individuals at other locations around the atoll. The largest individual they report at Rongelap was 60 cm; the largest individual I have measured at Enewetak was in the quarry on Enewetak Island and was 42 cm long.

It is possible that large individuals are not found in certain areas because transverse fission takes place at small and medium sizes. Certain environmental features could promote asexual reproduction, and in such locations only small to medium size animals would be found.

It is interesting that in the gutter at Ananij no new individuals appear to have come from the plankton. The same appears to be true for Bonham and Held's animals at Kabelle Island. All small individuals were very wide and probably represent fission products. A reasonable interpretation is that recruitment from the plankton is very unpredictable and asexual reproduction is an adaptation which permits the species to persist on a reef and span the periods of time during which no settlement from the plankton takes place. The apparent stability of the size structure at Ananij coupled with the estimated high loss rates and lack of recruitment from the plankton can be reconciled only by concluding that the rate of fission must be high.

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