AN ABSTRACT OF THE THESIS OF Randolph S. Rideout for the Master of Science in Biology presented April 18, 1975.

Title: Asexual Reproduction as a Means of Population Maintenance of the Coral Reef Asteroid *Linckia multifora* (Lamarck)

Approved: ______________________________
MASASHI YAMAGUCHI, Chairman, Thesis Committee

The asteroid *Linckia multifora* reproduces by autotomy of arms. Seven phases are recognized in its asexual reproductive cycle (autotomized arms; comets; counter-comets; post counter-comet I, II and III; and disc-parents). Individuals of the comet phase represent the highest percentage for population samples collected throughout the year at [illegible] reef flat, Guam. Cocos Lagoon and GabGab Beach population samples also demonstrated comet predominance. This indicates a continuous asexual reproduction through which populations of *L. multifora* are being maintained on the reefs of Guam.

ASEXUAL REPRODUCTION AS A MEANS OF POPULATION MAINTENANCE OF THE CORAL REEF ASTEROID *LINCKIA MULTIFORA* (LAMARCK)

by

RANDOLPH S. RIDEOUT

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGY

University of Guam

1975

TO THE GRADUATE SCHOOL:

The members of the Committee approve the thesis of RANDOLPH S. RIDEOUT presented April 18, 1975.

M. YAMAGUCHI, Chairman

J. A. MARCH, JR.

L. RAWLERSON

E. ROBBINS

ACCEPTED:

JAMES A. McDONOUGH
Dean of the Graduate School

ACKNOWLEDGMENTS

This project was supported by National Science Foundation grant GA-39948 to the University of Guam Marine Laboratory.

I would like to express my appreciation to Dr. F. W. E. Rowe, Australian Museum, for review and constructive criticism of this paper. I am indebted to Jerry Heslinga, with whom the field work was carried out. I also wish to acknowledge the help rendered by Steve Rice, Leslie Rice, Pat Beeman, Frank Cushing and Ted Tansy during the course of this study.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

INTRODUCTION

The voluntary autotomy of arms and regenerated "comet" forms derived from the cast-off arms has been noted in the asteroid, *Linckia multifora* (Hirota, 1895; Edmondson, 1935). In certain starfish, autotomy seems to be employed as an escape mechanism, the discarded rays eventually degenerating. However, this does not seem to be the case with *L. multifora*. Edmondson (1935) postulated that autotomy in the Hawaiian population of *L. multifora* was constant throughout the year and was a natural phenomenon representing a method of asexual reproduction. Grassle (1973) pointed out that asexual reproduction may be of great importance in the coral reef environment and that under optimal circumstances single genotypes may be favored.

Clark and Rowe (1971) showed that *L. multifora* is distributed throughout the Indo-Pacific. On Guam (Yamaguchi, in press) there are four species of starfish that are known to reproduce asexually by autotomy: *Echinaster luzonicus*, *Ophidiaster robillardi*, *Linckia guildingi* and *Linckia multifora*. Of these four autotomous species, *L. multifora* is the most abundant. This starfish is distributed in coral thickets of dead *Acropora* sp., under rocks on the reef flat, and on open substrate on the reef terrace, mostly on the lee coast of Guam. A series of specimens, including recently autotomized arms, comets in different stages of development and disc-parents with arms of various sizes, was represented in each collection obtained from various sites on Guam. "Disc-parent" is the name given here to *L. multifora* specimens with all arms autotomized.

The main purpose of this study was to determine the significance of autotomy and regeneration in *L. multifora* as a means of asexual reproduction and therefore as a mechanism for maintaining populations of this starfish on the coral reefs of Guam.

MATERIALS AND METHODS

A qualitative sampling of *L. multifora* was conducted on the Asan reef flat. I examined dead coral thickets of *Acropora* sp. by snorkeling and digging with a rock pick. Specimens representing all post-metamorphosis stages were sought. One hour "digs" were carried out twice a month from April, 1974 to March, 1975, except for April and December when only one dig per month was conducted. The *Acropora* sp. thickets where *L. multifora* is most commonly found were estimated from aerial photographs to cover approximately 5,600 m^2. Approximately 1 m^2 of this area was searched in each dig.

Specimens collected during the digs were carried to the University of Guam Marine Laboratory, fixed with formalin and removed after three days for drying. From these specimens, a size-frequency distribution for autotomized arms and comets of the Asan reef flat population was obtained. Specimens of the year-round collections of *L. multifora* which demonstrated autotomy were examined for a pattern in the sequence of autotomy to see if this process could account for maintenance of the population size.

Ten, 10-liter aquaria were used for regeneration and growth experiments. An additional 40-liter aquarium was maintained to accommodate fresh collections of *L. multifora* taken from Asan reef flat and was also used to isolate freshly autotomized arms and disc-parents for observation of their regeneration.

The regeneration experiment was carried out in two parts. In the first part, disc-parents and their autotomized arms were removed from the 40-liter aquarium after autotomy. These specimens were then measured and kept in a 10-liter aquarium where they were monitored to find the time (days) necessary for disc-parents to heal their wounds and for cast-off arms to regenerate a mouth. A maximum of four specimens of each form was kept per aquarium. In the second part of this experiment, only cast-off rays were removed from the 40-liter aquaria after their autotomy from a disc-parent. These arms were measured and kept in 10-liter aquaria for 40 days to determine rate of regeneration. A maximum of five arms was kept in each aquarium.

Growth increments for L. multifora were measured over a 30-day period. Four different-sized individuals were placed in each of ten aquaria. This provided sufficient numbers of organisms for statistical treatment, and made it possible to identify each individual. Initial size and size after 30 days were determined by the following technique. Photographs of the oral surfaces of variously sized comets were taken. The resulting black and white negatives were projected onto a 30-cm screen by a profile projector (Nikon, Model 6C), which enlarged the images to 20 times their original size. The images thus produced were measured. Autotomized arms and disc-parents used in regeneration experiments, and preserved arms and comets, were measured by the same technique but with images enlarged only to 10 times. All other forms of L. multifora were measured directly with calipers, using magnifying lenses. Preserved and freshly autotomized arms were measured along the length of the ambulacral groove. Preserved comets and comets used for determining individual growth increments were also measured along the ambulacral groove but from the center of the disc.

Food preferences of *L. multiform* are not clearly known, but these starfish are believed to be substrate feeders digesting microscopic organisms growing on surfaces of dead corals, *Acropora* sp. (Yamaguchi, in press). Food for starfish being monitored in regeneration experiments and in the growth study was provided weekly in the form of epibenthic microflora on dead *Acropora* skeletons taken from the area of *L. multiform* collections.

RESULTS

In the first part of the regeneration experiments, the time necessary for a disc-parent to heal its wound and begin arm regeneration was found to average 26 days, with a range of 19 to 30 days (N=10). Twenty-seven days were required for single cast-off arms to heal and develop a mouth, with a range of 20 to 30 days (N=10). In the second part of the regeneration experiments, single cast-off arms required forty days (from the day of autotomy) to regenerate comet arms with mean length of 1.3 mm (range 0.4 to 1.9 mm, N=10).

The "principal arm" of the comet demonstrated negative or positive growth during a 30-day period. The relationship of change of length in principal arm after 30 days to initial length of principal arm is plotted in Fig. 1. The coefficient of correlation (r) for these data is -0.38, thus demonstrating that little correlation exists between these two factors.

The data obtained from the 30-day growth period study indicated that the growth rate of regenerating comet arms was not correlated with the length of principal arms. These data are plotted in Fig. 2, and have a low correlation coefficient of -0.03. Likewise, the growth rate of regenerating comet arms and the growth rate of principal arms were independent of one another (r=0.01; Fig. 3). In view of this evidence a separate growth curve for regenerating comet arms was determined.

Initial length (L_1) and final length (L_2) for regenerating comet arms were converted to common logarithms. Common logarithms of final

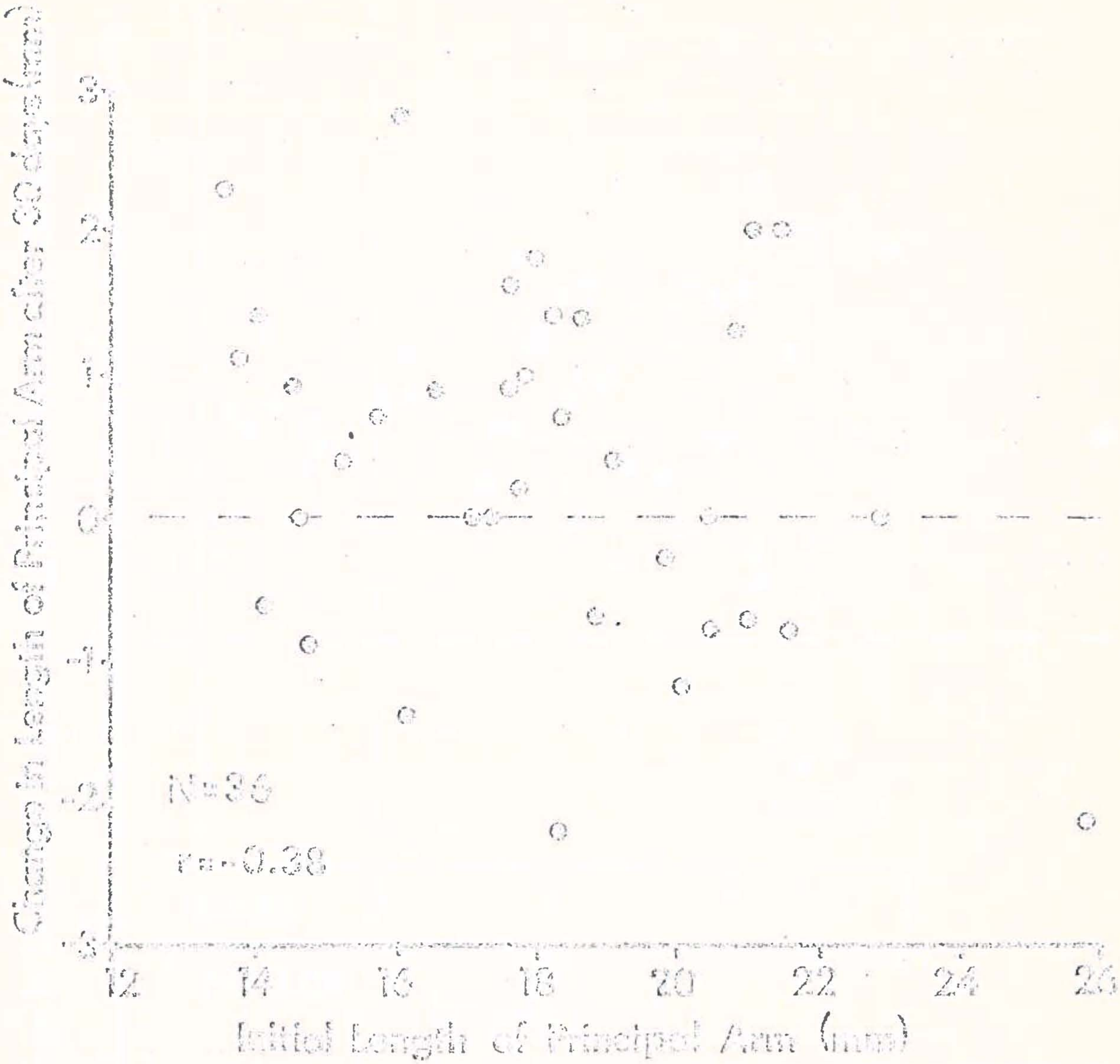

Fig. 1. Linckia multifora. Change in length of principal arm after 30 days (mm) versus initial length of principal arm (mm).

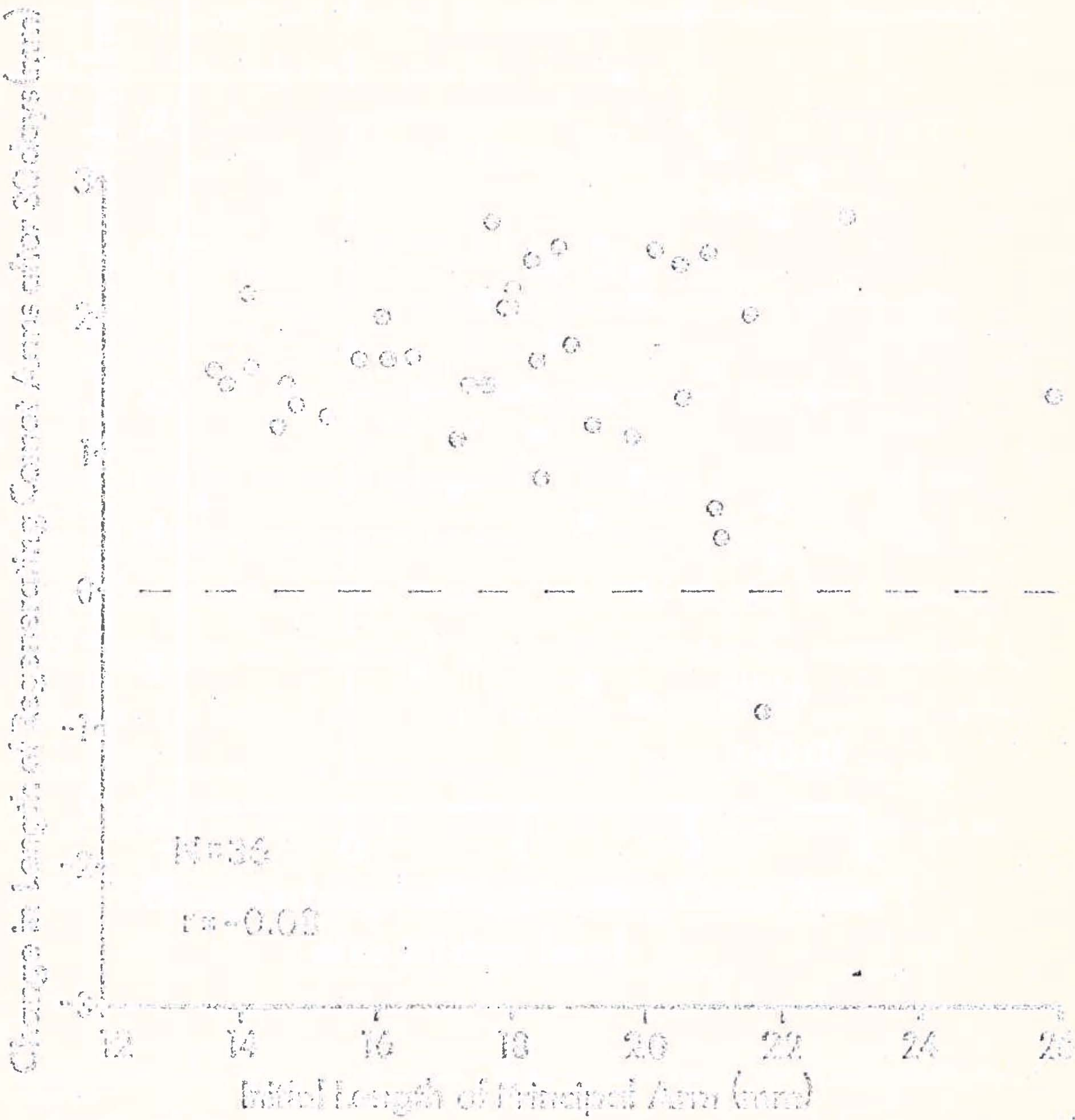

Fig. 2. Linckia multifora. Change in mean length of regenerating comet arms after 30 days (mm) versus initial length of principal arm (mm).

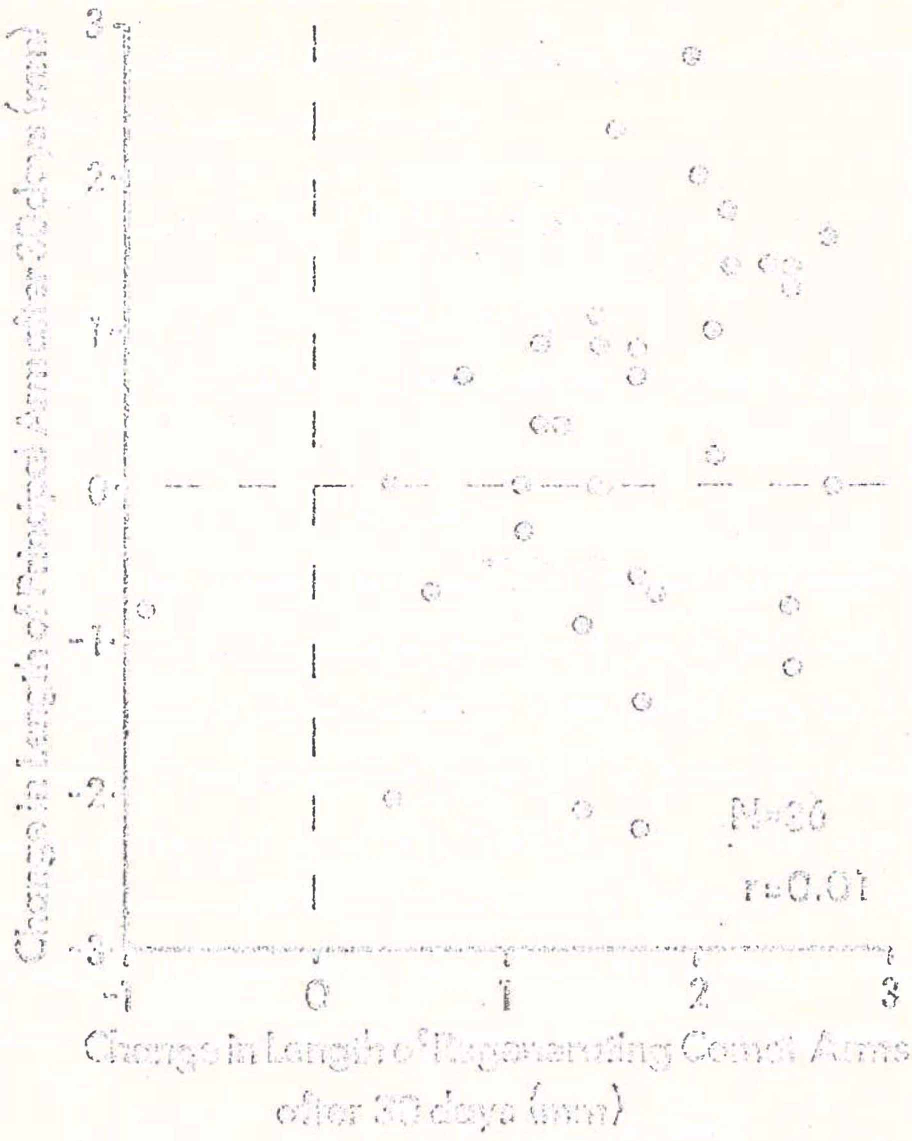

Fig. 3. Linckia multifora. Change in length of principal arm after 30 days (mm) versus change in mean length of regenerating comet arms after 30 days (mm).

length minus common logarithm of initial length and common logarithm of initial length are plotted in ordinate and abscissa, respectively (Fig. 4). The regression line is

$$Y = -0.280\ X + 0.351$$

where $Y = \log L_2 - \log L_1$, and $X = \log L_1$.

This is equivalent to a Gompertz growth equation of the form

$$L_t = L_\infty^{(1 - be^{-Kt})}$$

where L_∞ is the ultimate size estimate from the X-intercept of the regression line, calculated to equal 17.95 mm in this case. The growth parameter K was estimated as 0.328 per 30 days (or 1 month), and constant b was determined to be 1.39 by fixing the initial size L_0 equal to 1.3 mm at t equal to 1.3 months. The initial size was determined in regeneration experiments where it was found that 40 days (1.3 months) is required for autotomized single arms to reach 1.3 mm in mean arm radius. The hypothetical growth curve equation is:

$$L_t = 17.95^{(1 - 1.39e^{-0.328t})}$$

where one time unit is 30 days. This growth curve is illustrated in Fig. 5. Data from regeneration experiments are included in this growth curve to illustrate the transition from autotomized arm to young comet. An autotomized arm heals and develops a mouth during the interval t=0 to t=0.9. The healed arm reaches a mean length of 1.3 mm by t=1.3. From this point (t=1.3, L=1.3) the Gompertz growth equation is used to illustrate a mean growth curve for regenerating comet arms.

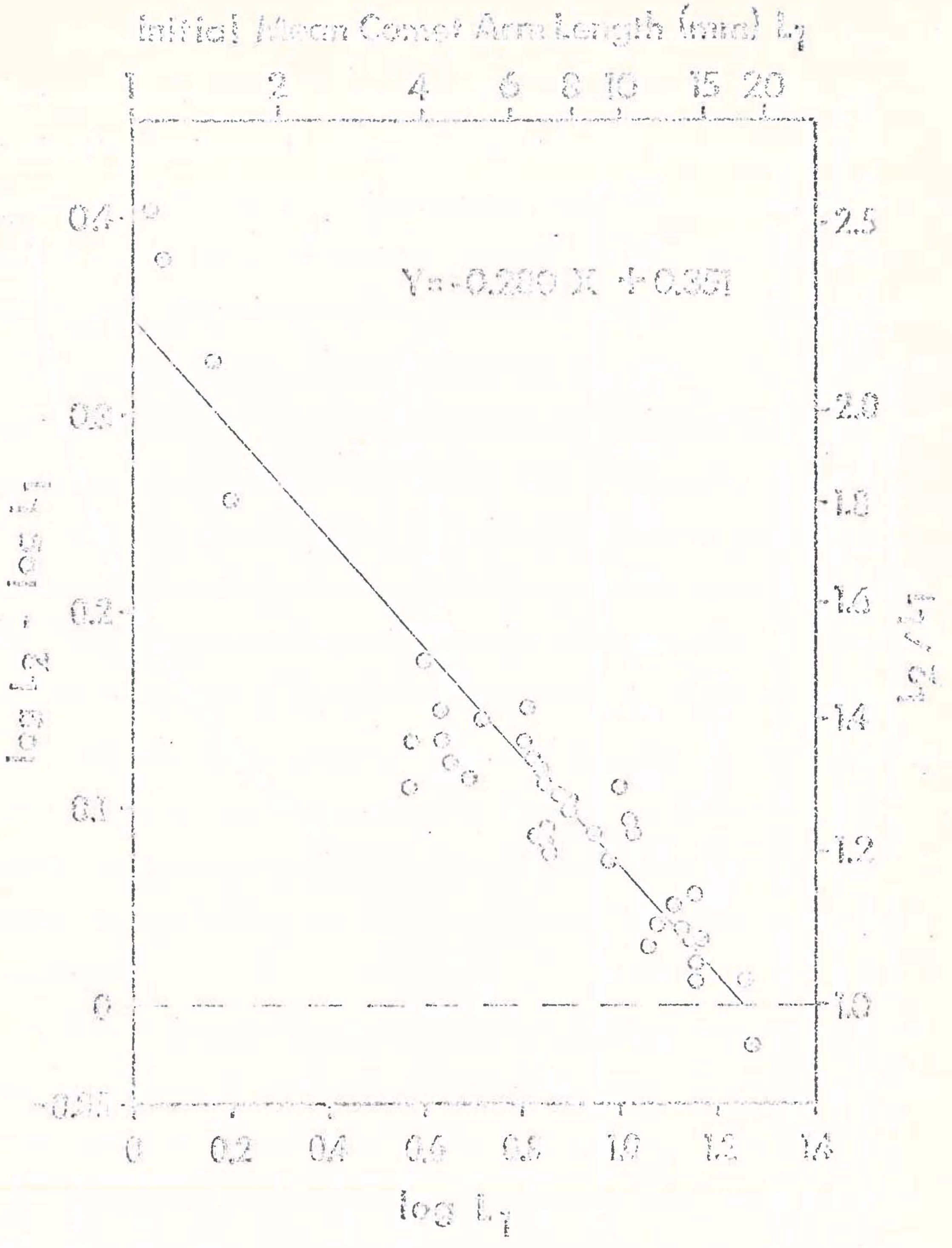

Fig. 4. Linckia multifora. Growth data of regenerating comet arms. Abscissa: common logarithm of initial comet arm length (mm); ordinate: common logarithm of final minus common logarithm of initial mean comet arm length after 30 days. For further explanation see the text.

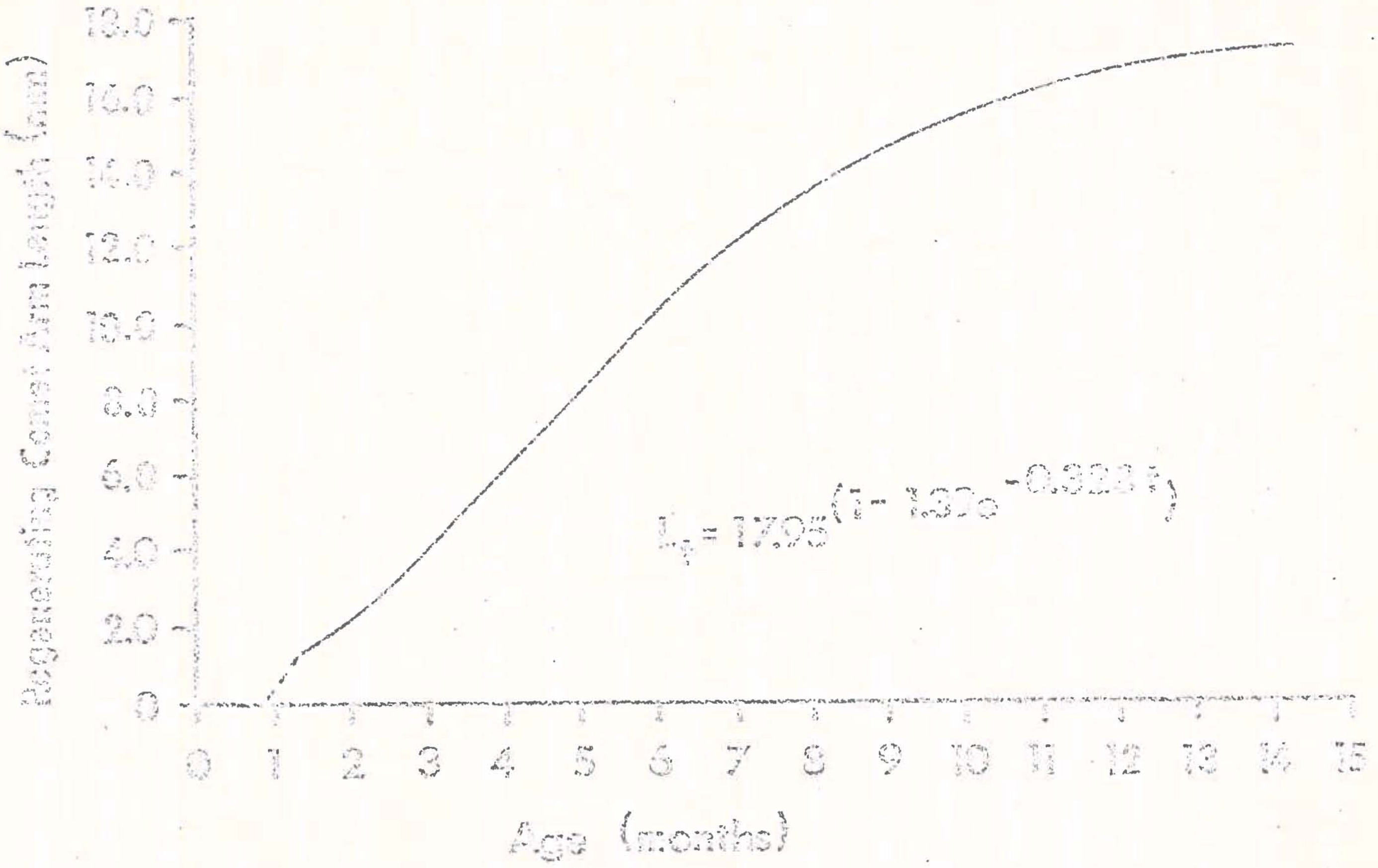

Fig. 5. Linckia multifora. Growth curve based on mean regenerating comet arm length (mm). The interval from t=0 to t=1.3 is a period required for regenerating comet arms to reach 1.3 mm in mean arm length, as determined from regeneration experiments. The Gompertz growth curve represents the regression line of Fig. 4, where the initial size is taken as 1.3 mm at age 1.3 months. For further explanation see the text.

Seven phases of the asexual cycle were identifiable from the Asan reef flat population samples and are illustrated in Fig. 6. Through observation of regeneration experiments and comet specimens it was discovered that two madreporites were always developed, one on either side of the principal arm. Because of this consistency of madreporite positioning, it was possible to utilize a numbering system for L. multifora arms. The principal arm was labelled No. 1 (Fig. 6A and 6B); other arms were labelled with successive numbers in a counter-clockwise order on the aboral surface (Fig. 6A and 6B).

Specimens collected from the Asan reef flat were grouped each month according to phase of reproductive cycle, as shown in Table I. The total number of starfish found in 22 digs conducted over 12 months was 801. Individuals having four arms (N=28) or six arms (N=4) and those infested by the parasitic gastropod Stylifer sp. (N=5) comprised less than 5% of the total population and were not treated in this study. The mean number of individuals and standard deviation per sample for the 22 digs was 36.4 ± 15.5, after exclusion of the forms mentioned.

Size-frequency histograms of the length of autotomized arms for each month (Fig. 7) indicated the continual occurrence of autotomized arms except for the months of October and December. The recently autotomized arms were 8 to 24 mm in length and their total number in the population was 67 of 801 individuals (8.4%) for all samples. The absence of cast-off arms in the above mentioned two months can be attributed to small numbers of individuals and the relatively short duration of the autotomized arm stage.

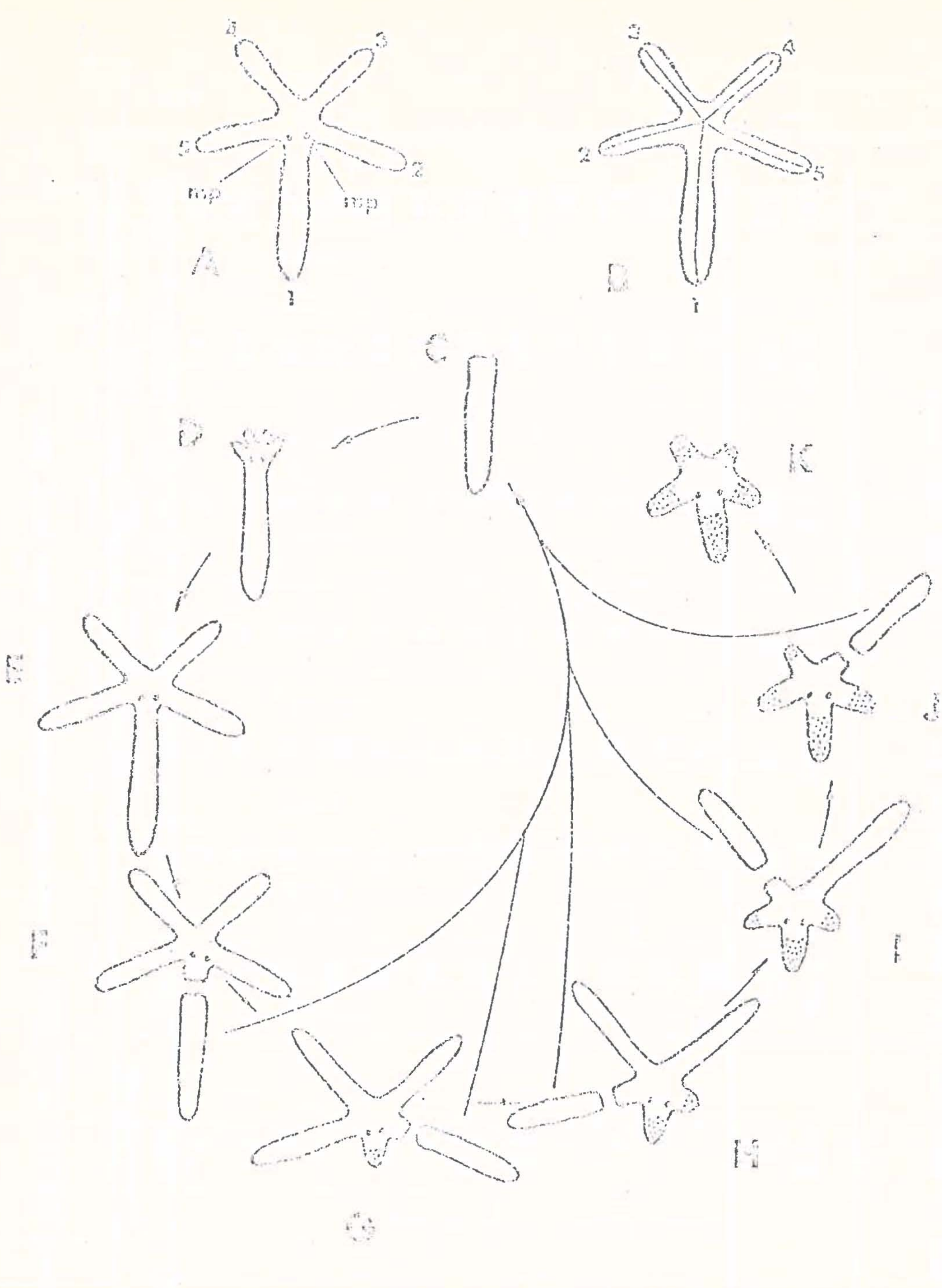

Fig. 6 Linckia multifora. Schematic asexual reproductive cycles. A, comet, aboral surface, showing two madreporites (ap) and the numbering system; B, comet, oral surface, with lines indicating ambulacral groove; C, autotomized arm; D, young comet; E. advanced comet; F, counter-comet (c-c); G, post c-c I; H, post c-c II; I, post c-c III; J, disc-parent; and K, disc-parent demonstrating regeneration in all arms. Counter-comet (F) casts off either arm 2 or 5 with equal occurrence (only arm 2 autotomy is drawn here). Illustrated phases beyond post c-c I represent one possible sequence of autotomy.

Table I. Year-round *Linckia multifora* population structure of Asan reef flat.

Month	Man-hours[a]	Autotomized arms	Comets	c-c[b]	Post c-c I	Post c-c II	Post c-c III	Disc-parents	Total
Apr. 1974	1	6	14	1	1	0	1	4	27
May	2	9	23	5	3	2	3	20	65
June	2	8	28	4	0	0	1	14	55
July	2	1	42	9	2	1	2	27	84
Aug.	2	10	48	11	2	4	2	33	110
Sept.	2	1	19	3	1	1	1	11	37
Oct.	2	0	33	13	0	2	4	12	64
Nov.	2	13	31	26	5	4	3	26	108
Dec.	1	0	9	5	0	1	1	4	20
Jan. 1975	2	6	36	9	3	2	2	13	71
Feb.	2	9	39	18	4	4	2	20	96
Mar.	2	4	30	13	3	4	3	7	64
Total	22	67	352	117	24	25	25	191	801
Percentage		8.4	43.9	14.6	3.0	3.1	3.1	23.8	100

[a]One man-hour search (a one hour dig) was estimated to cover 1 m^2 in each case.

[b]c-c is the abbreviation used for counter-comet.

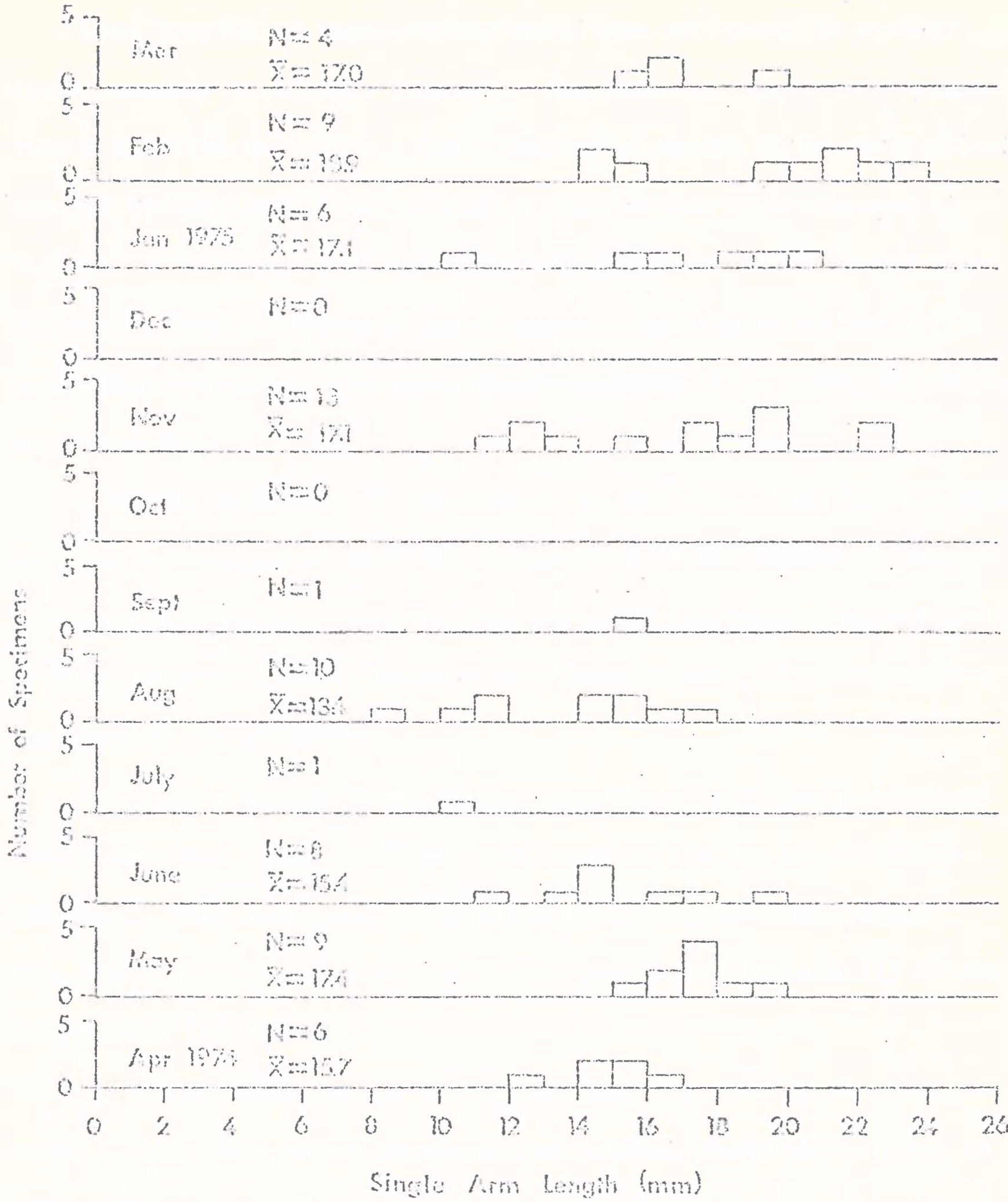

Fig. 7. Linckia multifora. Monthly size-frequency histograms of the length (mm) of autotomized arms.

Mean lengths of regenerating comet arms are shown in monthly size-frequency histograms (Fig. 8). Regenerating comet arms ranged from 1 to 21 mm in length. Total number of comets in the population was 352 of 801 individuals (43.9%). This series of histograms suggests that there is no distinctly dominant age (size) group for the comet stage. Figures 5 and 6 indicate that there is little seasonality in autotomous asexual reproduction in *L. multifora* on the Asan reef flat.

From the regeneration experiments it was found that regeneration rate was a function of time and independent of the number of arms autotomized. Arm regeneration rate was thus interpreted as a function of time after autotomy. Knowing this relationship, I constructed a sequence of arm autotomy.

There is a marked trend in the sequence of autotomy. Advanced comets cast off their principal arm first in 111 out of 117 cases examined. This condition resulted in the "counter-comet" stage (Fig. 6F), whose overall number in the population totaled 117 of 801 individuals (14.6%).

In the specimens which had autotomized two arms (post counter-comet I, Fig. 6G), after developing as comets, there was a trend of autotomizing the arms adjacent to the principal arm (arm 2 or 5). The post counter-comet I phase was represented by 24 individuals (3.0% of the population) and of this sample 20 individuals (83%) of the specimens demonstrated either arm two or five to be the second arm autotomized. Based on regeneration rates, I concluded that the principal arm was the first arm autotomized in all cases.

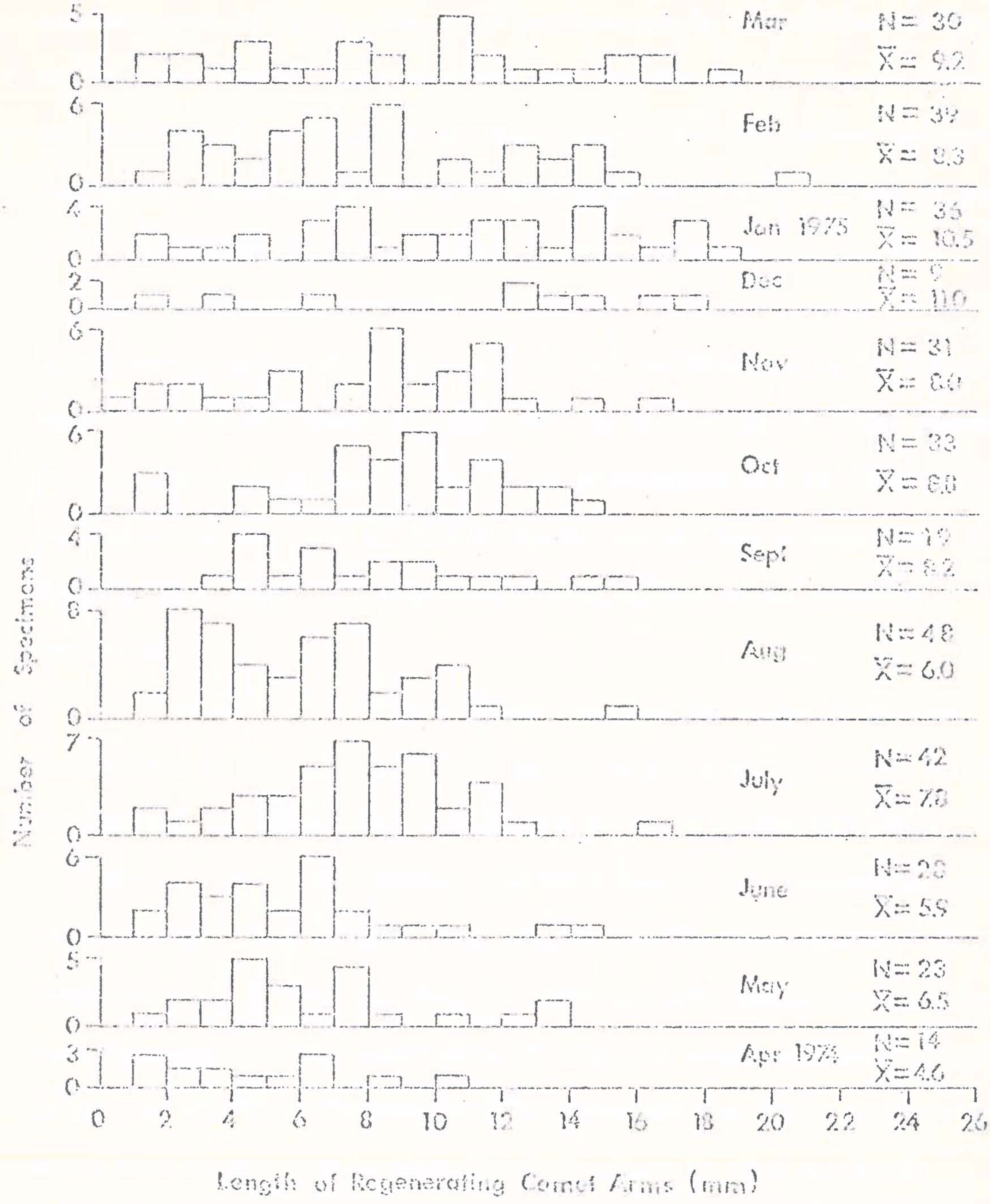

Fig. 8. Linckia multifora. Monthly size-frequency histograms of the mean length (mm) of regenerating comet arms.

Specimens which have autotomized three arms, designated as "post counter-comet II" (Fig. 6H), were represented by 25 individuals (3.1% of the population). The principal arm was observed to be the first arm autotomized in 23 (92%) of these cases. For the second arm autotomized in these specimens, 22 individuals (88%) cast off either arm 2 or 5. They demonstrated no trend for the third arm autotomized.

Those specimens with four arms autotomized are designated as "post counter-comet III" (Fig. 6I) and are represented by 25 individuals (3.1% of the total population). In this group 24 (96%) showed the principal arm to be the first one autotomized and 17 (68%) demonstrated either arm two or five to be the second arm autotomized. No trend of sequential autotomy could be discerned beyond autotomy number two.

In total, the principal arm was observed to be the first arm autotomized in 182 of 191 (95.3%) cases, and the second arm to be autotomized was either arm two or five in 59 of 74 (79.7%) cases.

For the entire collection, the mean length of regenerating arms of comets is 7.7 mm (N=352), the mean length for regenerating arms of counter-comets is 14.8 mm (N=111). The mean arm length for the three remaining regenerating comet arms of the post counter-comet I stage is 19.4 mm (N=24). The mean length of the two remaining regenerating comet arms of the post counter-comet II stage is 19.9 mm (N=25) (Fig. 9). This demonstrates that original comet arms do continue to grow during the advanced stages where autotomy of growing arms takes place.

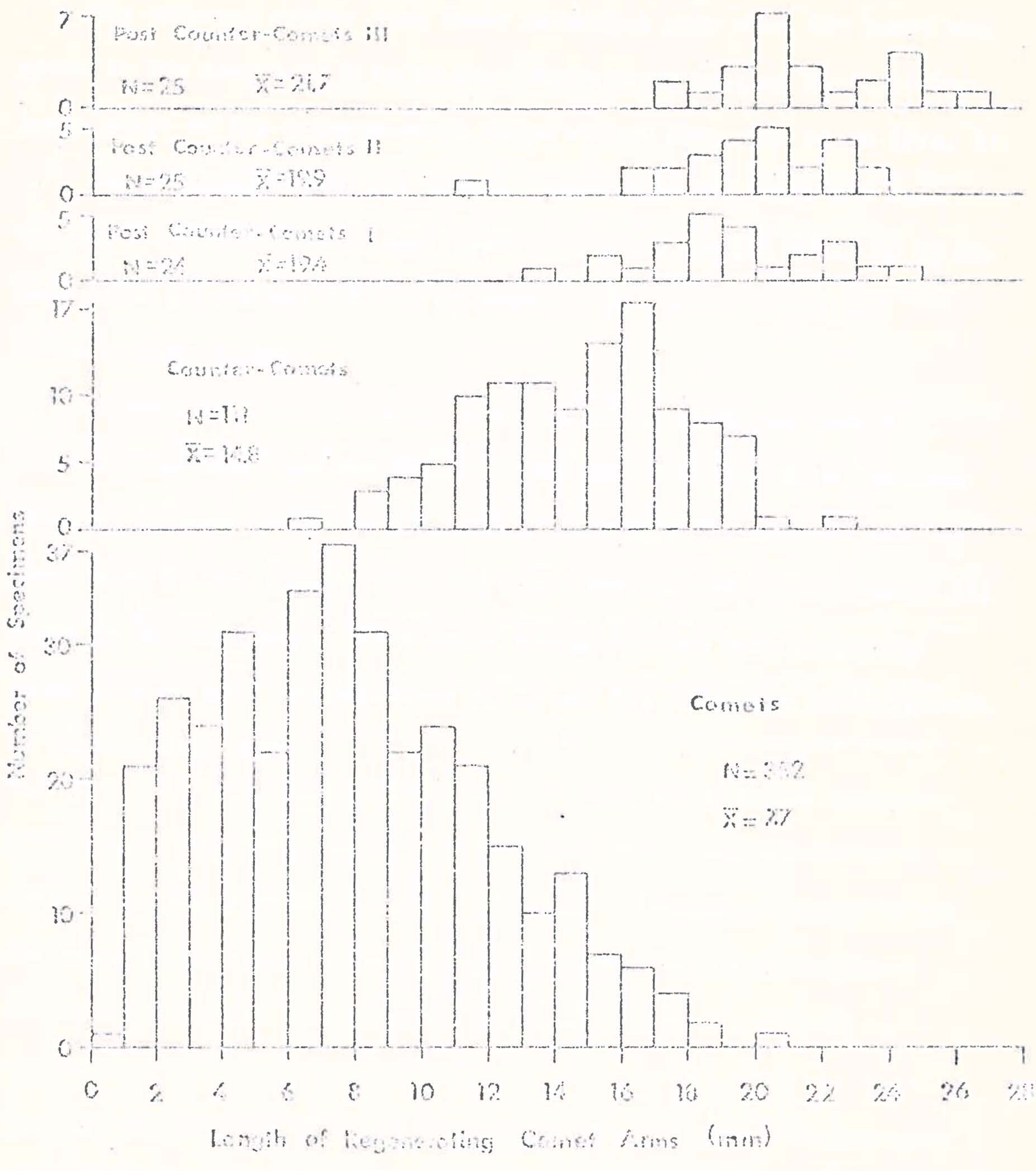

Fig. 9. Linckia multiflora. Size-frequency histograms of mean length (mm) of regenerating comet arms for comets, counter-comets, post counter-comets I, II and III. Six counter-comets not demonstrating principal arm autotomy are excluded.

The advanced comets cast their principal arms when the comet arm grows to the length of 6 to 23 mm. There is an evident shift of the comet stage to the counter-comet stage within this size range (Fig. 9).

Counter-comets cast off either their second or fifth arm when the three remaining comet arms grow to the mean length of 13 to 25 mm. This shift is evident but not as dramatic as between comet and counter-comet stages.

Between post counter-comet I and II there is little change between remaining regenerating comet arm mean values. Also, between the mean of regenerating comet arms of post counter-comet II and the mean value of the single remaining comet arm of post counter-comet III there is little change. This small change between mean remaining comet arms of post counter-comet I, II and III indicates little growth of regenerating arms.

DISCUSSION AND CONCLUSION

The small percentage (8.4%) of freshly autotomized arms in the population sampled can be attributed to the relatively short regeneration period (40 days) during which such arms develop to young comets.

Comets constitute the highest percentage (43.9%) of the population. This high percentage of the population can be attributed to the long growth period (4 to 12 months) needed for regenerating arms to develop to the size required for autotomy. The second most common individuals are disc-parents, which will be discussed later.

Counter-comets represent the third largest portion of the population at 14.6%. This large number in the population can be attributed to input from comets and growth of arms until second autotomy. Post counter-comets I, II and III represent the three lowest percentages of the population: 3.0, 3.1, and 3.1 percent, respectively. The small percentage of the population represented by these three stages can probably be attributed to a short interval between autotomies 2, 3 and 4.

It was observed that all arms autotomized between counter-comet and disc-parent stages are capable of re-entering the reproductive cycle. Thus each autotomized arm has the potential of contributing a minimum of 5 arms to the asexual cycle before it is termed a disc-parent. Disc-parents, which represent 32.8% of the population, have been observed to autotomize regenerated arms. These cast-off rays re-enter the asexual cycle. However, it is not known how many times a disc-parent may continue this sequence of autotomy and regeneration. Neither is it known to what extent mortality may contribute to the population structure on the Asan reef flat.

Two other population samples from elsewhere on Guam were examined to see whether or not they demonstrated population structures similar to that on the Asan reef flat. The first sample was taken from GabGab Beach in April, 1974. L. multifora was found at depths of 1 to 3 m on the reef front. A population sample of 76 was obtained after approximately 4.5 man-hours' search. A second population sample of 61 was obtained from Acropora sp. thickets in Cocos Lagoon in November, 1974, after about 6 man-hours' search. Population structures from Cocos Lagoon, GabGab Beach and Asan reef flat are compared in Table II. It can be seen from this table that comets represent the largest percentages of all three populations; disc-parents represent the second largest and counter-comets the third. The four other phases make up relatively small percentages of the populations. These data suggest that other populations of L. multifora on the reefs of Guam have similar population structures and are reproducing in a manner similar to those at Asan.

Edmondson (1935) in Hawaii found that a period of 49 days was required for the formation of a mouth in cast-off arms of L. multifora approximately 100 mm long. On Guam, arms with a mean length of 14.7 mm require 27 days for mouth formation. Arm regeneration rate in Guam specimens does not appear to be related to arm size. Thus further investigation may be necessary to account for differences in arm size and regeneration rate between these geographically distinct populations.

Table II. Population structures of <u>Linckia multifora</u> at three locations on Guam.

Location	Man-hours	Autotomized arms	Comets	c-c[a]	Post c-c I	Post c-c II	Post c-c III	Disc-parents	Total
Asan reef flat	22	67	352	117	24	25	25	191	801
		(8.4%)	(43.9%)	(14.6%)	(3.0%)	(3.1%)	(3.1%)	(23.8%)	(100%)
GabGab Beach[b]	4.5	11	33	12	3	6	1	10	76
		(14.5%)	(43.4%)	(15.8%)	(3.9%)	(7.9%)	(1.3%)	(13.2%)	(100%)
Cocos Lagoon	6.0	2	28	5	5	4	1	16	61
		(3.3%)	(45.9%)	(8.2%)	(8.2%)	(6.6%)	(1.6%)	(26.2%)	(100%)

[a] c-c is the abbreviation used for counter-comets.

[b] Four and six rayed starfish representing 6.1 and 1.2 percent, respectively of the population are not included in this table.

Edmondson (1935) noted a high mortality rate of cast-off L. multifora arms kept in aquaria. He also suggested that the number of free rays and comets was not commensurate with the number of regenerating rays of living specimens and suspected this was due to high mortality rate and cryptic behavior in freshly isolated rays.

Davis (1967) also stated that survival of autotomized L. multifora arms in Hawaii was very low. In two experiments with autotomized arms kept in aquaria, only 15 of 64 arms survived after 21 days and only 2 of 60 arms survived after 14 days. On Guam, high mortality was not observed in my tanks. From regeneration experiments 100% survivorship was obtained for 10 autotomized arms kept for 30 days and another 10 arms kept for 40 days. This indicates that autotomized arms from L. multifora on Guam may not have as high a mortality rate as the L. multifora in Hawaii.

Little information on sexual reproduction in L. multifora has been published. Hirota (1895), working with L. multifora from the Bonin Islands, found one comet containing eggs in the principal arm, while another comet showed no gonad development. Edmondson (1935), noted that autotomy in the Hawaiian population of L. multifora was a year-round occurrence regardless of the spawning season, which extended from December to May. Mortensen (1938) stated that L. multifora in the Red Sea had a high degree of reproduction by autotomy. This same population demonstrated sexual reproduction by eggs, and pelagic larvae were noted from the middle of May to September. Mortensen did not find gametes in small "regenerating arms" (apparently equivalent to my autotomized arm phase), but did find gonads in the basal portions of the arms remaining on the parent organism (designated disc-parent in this paper).

I induced spawning in three individuals during July, 1974 (from a sample of 10 disc-parents) by injecting them with 1 cc of 10^{-5} molar solution of 1-methyladenine. The number of eggs spawned by each female was estimated to be about 200. Individuals of other samples collected monthly during the one-year period (N=75) did not spawn after the injection of 1-methyladenine.

The juvenile of L. multifora has yet to be raised in culture or found in nature. Mortensen (1938) raised L. multifora larvae in the laboratory to the brachiolaria stage, but did not observe metamorphosis to the juvenile form. The appearance of L. multifora juveniles is thus a matter of speculation.

It is likely that juvenile L. multifora have five arms of nearly equal length prior to the first autotomy. The smallest freshly autotomized arm found during extensive searches at Asan reef flat was 8.8 mm long, suggesting that arms must be near this size before autotomy can occur. Juvenile L. multifora, then, probably exhibit five similarly sized rays between approximately 1 and 8 mm in length. The Asan reef samples contained no individuals of this description, suggesting either that my sampling technique needs to be modified, or that there was an actual lack of juveniles in this area.

Recruitment by sexual reproduction may be a sporadic phenomenon in this asteroid and juvenile L. multifora may not be recognizable at present in the Asan reef flat population. However, this population and others sampled on Guam are successfully maintaining themselves through asexual reproduction by autotomy.

LITERATURE CITED

Clark, A. M., and F. W. E. Rowe. 1971. Shallow-water Indo-West Pacific echinoderms. British Museum Pub. No. 690. 238 pp.

Davis, L. D. 1967. The suppression of autotomy in *Linckia multifora* (Lamarck) by a parasitic gastropod, *Stylifer linckiae* Sarasin. Veliger. (9)2:343-346.

Edmondson, C. H. 1935. Autotomy and regeneration in Hawaiian star-fishes. B. P. Bishop Mus. Occas. Pap. 11:3-20.

Grassle, J. F. 1973. Variety in coral reef communities. *In* C. A. Jones and R. Endean eds. Biology and Geology of Coral Reefs, Vol. 2, Biol. 1. 276 p. Academic Press, Inc. New York.

Hirota, S. 1895. Anatomical notes on the comet of *Linckia multifora*, Lamarck. Zool. Mag. 7:67-76.

Mortensen, T. 1938. Contributions to the study of the development and larval forms of echinoderms. IV. K. Danske Vidensk. Selsk. Skr. (Naturv. Math.) (9)7:1-59.

Yamaguchi, M. (In Press). Coral reef asteroids of Guam. Biotropica.